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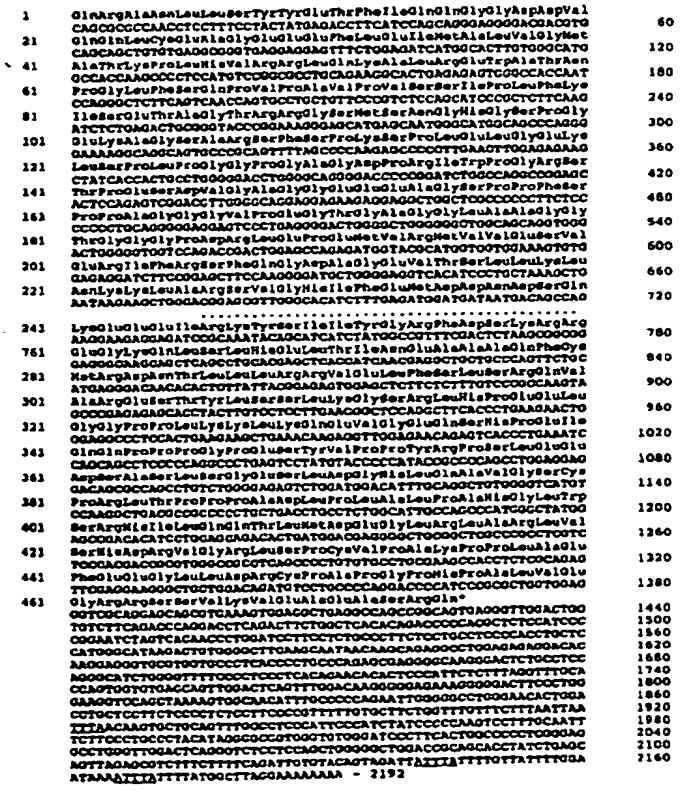
(54) Title: COMPOSITIONS AND METHODS USEFUL IN THE DETECTION AND/OR TREATMENT OF CANCEROUS CONDITIONS

(57) Abstract

Diagnostic and therapeutic compositions and methods which target a melanoma associated delayed early response (MADER) gene and its expression products are described. Specifically, the invention relates to the production, characterization and use of monoclonal antibodies capable of specifically binding to an approximately 55 kD MADER protein which is overexpressed in human malignant melanomas and other human cancerous tissue. Such antibodies are able to detect overexpressed MADER in cultured cells and frozen or paraffin-embedded sections of human biopsy material. The MADER protein, fragments or analogs thereof, or its gene in a vector suitable for a DNA vaccine, are employed as anti-cancer immunogens in the immunotherapeutic treatment of malignant melanoma and other cancerous conditions. Similarly, MADER polynucleotides are used herein in various cytological methods for detecting cells which overexpress MADER. MADER mRNAs are used as targets in antisense and ribozyme therapies directed at inhibiting the expression of MADER in a treated subject.



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COMPOSITIONS AND METHODS USEFUL IN THE DETECTION AND/OR TREATMENT OF CANCEROUS CONDITIONS

Technical Field:

The present invention relates generally to diagnostic and therapeutic compositions and methods which target a melanoma associated delayed early response (MADER) gene and its expression products. More particularly, the invention relates to the diagnostic use of anti-MADER molecules, and the use of MADER polynucleotides and polypeptides in the diagnosis and treatment of malignant melanomas and other cancerous conditions.

Background of the Invention:

A number of genetic events that are involved in the pathogenesis of cancer have been identified and partially characterized in both inherited and acquired human neoplastic disease. It is now generally recognized that neoplasms arise through a series of genetic changes in specific oncogenes. Since these changes are intimately associated with tumor progression, oncogenes provide useful markers for cancer detection, and likewise offer potential targets for therapeutic intervention. Particularly, the detection of DNA alterations in blood and cytologic samples enables the rapid and sensitive diagnosis of a number of neoplasms. This ability has revolutionized the approach to cancer diagnosis, health screening and patient assessment in modern health care.

The changes in gene expression which accompany the development of malignant tumors result in both early events, such as deregulation of cell

growth, and later events that lead to metastasis formation (Mendelsohn et al. (1995) *The Molecular basis of Cancer*, WB Saunders, Philadelphia). For example, the histopathologic progression of colorectal carcinoma from adenoma to carcinoma has been carefully studied, and a number of steps in the progression have now been correlated with specific genetic events that appear to drive the progression pathway. Thus, it is now known, for example, that activation of proto-oncogenes such as K-*ras* and the inactivation of a tumor suppressor gene such as *APC* occur early in the pathway. Late events include the loss of chromosome 17p among other chromosomal alterations.

Other chromosomal alterations leading to changes in gene expression have implicated a number of molecules in the early events of tumorigenesis (Vogelstein et al. (1993) *Trends Genet.* 9:138-141). Many of the implicated molecules appear to function in DNA repair and growth regulation, and have been found to be mutated or deleted in a variety of different carcinomas. For example, in human cutaneous melanoma, alterations in the expression of cell and matrix adhesion molecules accompanying tumor progression and metastasis development have been extensively described. *See, e.g.*, Albelda et al. (1990) *Cancer Res.* 50:6757-6764; Johnson et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:641-644; Klein et al. (1991) *J. Invest. Dermatol.* 96:281-284; Lehmann et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9891-9895; Martin-Padura et al. (1991) *Cancer Res.* 51:2239-2241. However, with the exception of p53 (Albino et al. (1994) *Melanoma Res.* 4:35-45; McGregor et al. (1993) *Br. J. Dermatol.* 128:606-611), most of the molecules involved in early changes in carcinomas do not seem to play a role in the development of melanomas.

A number of investigations have attempted to identify specific genetic events that are involved in

the progression of melanoma. One such study has associated mutations in the cyclin dependent kinase 4 inhibitor p16 gene with some cases of familial melanoma (Liu et al. (1995) *Oncogene* 11:405-412). Further, even though mutations appear to be rare in sporadic tumors *in vivo* (Ohta et al. (1994) *Cancer Res.* 54:5269-5272), a loss of p16 expression has been observed in a number of metastatic lesions (Reed et al. (1995) *Cancer Res.* 54:5269-5272). Thus, the genetic basis of melanoma progression has remained elusive, and our understanding of the mechanisms involved in growth de-regulation in melanomas remains sparse.

Summary of the Invention:

The present inventors have discovered a novel nuclear protein that is associated with growth alterations in malignant melanomas and other cancerous conditions. The subject protein is the "melanoma associated delayed early response" protein, and termed "MADER" herein. Studies described below establish that MADER mRNA is rapidly induced in a wide range of cells following exposure to mitogens or growth factors. This induction requires protein synthesis indicating that MADER belongs to the set of growth factor dependent delayed early response genes that are induced during the G1 stage of the cell cycle (Nathans et al. (1988) *Cold Spring Harbor Symposia on Quantitative Biology* LIII:883-900).

The MADER gene is highly conserved, as cross-hybridizing DNA sequences have been observed in species as diverse as Rhesus and *S. cerevisiae*. The predicted MADER expression product is approximately 55 kD, and is characterized as having two proline rich domains, 15 potential phosphorylation sites, a nuclear localization signal, and multiple S(T)PXX motifs that are characteristic of regulatory DNA binding proteins.

Immunological studies presented below confirm that MADER is localized to the nucleus. These features suggest that MADER participates in growth regulation.

Although Northern analyses indicate that low-level expression of MADER is ubiquitous among most types of cells, MADER expression is only rarely detectable in normal tissue. In contrast, strong nuclear anti-MADER staining has been observed in all malignant melanomas tested, and has been observed in several breast carcinomas. Further, the fact that only one of six benign melanocytic nevi examined showed evidence of MADER expression suggests that over-expression of the MADER protein may be associated with the malignant transformation of melanocytes. Thus, the MADER gene and its expression product present novel targets for malignant melanoma, and other cancer diagnostics and therapeutics.

Accordingly, the present invention provides diagnostic and therapeutic compositions and methods which target the MADER gene and its expression product. In one embodiment, monoclonal antibody molecules capable of specifically binding to the approximately 55 kD MADER protein are provided. The subject anti-MADER monoclonal antibodies are used herein in immunohistochemical methods of detecting malignant melanoma cells, or other cancerous cells, in a tissue sample. Particularly, methods are described wherein tissue samples suspected of containing cancerous cells, such as malignant melanoma cells, are exposed to anti-MADER monoclonal compositions, and the presence or absence of bound antibodies on the tissue sample is determined as an indication of the presence or absence of a cancerous condition, such as a melanoma malignancy. Visualization of positive binding events is effected by detectably labeling either the anti-MADER monoclonals (direct detection),

or by detecting secondary molecules capable of binding the anti-MADER molecules (indirect detection).

In another embodiment, MADER polynucleotides are targeted in cytological analysis methods for detecting a cancerous condition, such as a melanoma malignancy, in a tissue sample. Particularly, methods are provided for detecting the presence of cells that over-express the MADER gene. Detectably labeled oligonucleotide probes are provided which comprise a nucleotide sequence that is complementary to a region of mRNA transcribed from the MADER gene. Mixed-phase hybridizations are conducted by incubating the subject probes with cell lysates (obtained from a selected tissue sample) under suitable conditions. A detection step is then carried out to detect the presence of any labeled probe on the substrate. In this manner, a signal can be obtained which is indicative of the presence or absence of cells in the tissue sample which over-express the MADER gene. In addition, *in-situ* hybridizations can be carried out by incubating the subject probes with immobilized tissue sections (obtained from a selected tissue sample) under suitable conditions.

In related embodiments, methods are provided for visualizing the presence or absence of a translocation of MADER in cellular genomes using fluorescence *in situ* hybridization. MADER translocations are thought to give rise to over-expression of MADER in malignant melanoma cells and other cancerous cells; and, in this manner, these *in situ* hybridizations can be used to detect a cancerous condition, such as malignant melanoma, in a tissue sample.

In yet other embodiments of the invention, therapeutic compositions containing MADER immunogens are provided for use in immunizing a subject having a cancerous condition such as malignant melanoma. The

MADER immunogens can be obtained from either MADER polypeptides or polynucleotides. In another therapeutic application, MADER antisense molecules are used in tumor treatments. More particularly, antisense oligonucleotides capable of selectively binding to target MADER sequences are provided. The antisense oligonucleotides generally comprise synthetic nucleic acid sequences that bind specifically and predictably to complementary regions of MADER mRNA, thereby inhibiting MADER protein biosynthesis. Other related therapeutics include ribozymes that are capable of degrading MADER mRNAs.

These and other embodiments of the invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

Brief Description of the Figures:

Figure 1 depicts the nucleotide sequence (SEQ ID NO: ___) of human MADER cDNA for the Drop9 variant. The deduced amino acid sequence (SEQ ID NO: ___), corresponding to the longest open reading frame (coding for a protein of 474 amino acids) is also shown. Two potential translation starts are indicated in the Figure (with filled circles) at nucleotides 104 and 119. A single putative N-linked glycosylation site is indicated by brackets (amino acid 237), and 9 potential DNA binding units are indicated within boxes. A bipartite nuclear localization signal is indicated by boxed amino acids with an overlying dotted line. The 3' untranslated region has one polyadenylation site (AATAAA) at position 2160. Located within this 3' region are three ATTTA repeats which have been underlined in the Figure.

Figure 2 depicts the nucleotide sequence (SEQ ID NO: ___) of human MADER cDNA for the Drop8 splice variant. The deduced amino acid sequence (SEQ ID NO: ___) is also shown. The nucleotide sequence of

the Drop8 variant lacks 192 base pairs as compared to the Drop9 variant. These missing 192 base pairs are underlined and italicized in the Figure.

Figure 3 depicts the mRNA sequence (SEQ ID NO.:___) of yet another MADER variant. A full-length amino acid sequence (SEQ ID NO.:___) of the MADER variant, having 525 residues, is also shown. The amino acid sequence (SEQ ID NO.:___) of a 329 residue MADER polypeptide encoded by yet a further splice variant is also shown in the figure.

Figure 4 depicts the results from a genomic Southern blot analysis of the MADER gene. Genomic DNA from EBV-transformed B cells of a normal individual was digested with the indicated restriction endonucleases. The positions of the molecular weight markers are indicated on the left of the gel.

Figure 5 depicts the hybridization of human cDNA with DNAs isolated from a variety of different species (Genomic Zoo blot). The gel contains *Eco*RI digested DNA from human (lane 1), Rhesus monkey (lane 2), rat (lane 3), mouse (lane 4), dog (lane 5), cow (lane 6), rabbit (lane 7), chicken (lane 8), *S. cerevisiae* (lane 9). Arrowheads in the Figure indicate the location of the weakly hybridizing bands in the rat (lane 3) and the chicken (lane 8). The blots were hybridized with [^{32}P]-labeled complete Mader cDNA.

Figure 6 depicts induction of Mader mRNA production in Mel JuSo cells by exposure to 20% serum (FCS) or phorbol 12-myristate 13-acetate (PMA). The cells were harvested at the indicated times following stimulation. 20 μg total RNA was used, and the blots were hybridized with [^{32}P]-labeled complete Mader cDNA, or with oligonucleotides detecting c-*fos* or GAPDH. All blots were analyzed by autoradiography and densitometry.

Figure 7 depicts induction of Mader mRNA production in lymphocytes stimulated with PHA. Freshly isolated PBMC were exposed to PHA for the indicated times. GAPDH was used as control for RNA loading. All blots were analyzed by autoradiography and densitometry.

Figure 8 depicts the effect of cycloheximide on the induction of Mader mRNA production in Mel JuSo cells. The cells were grown to confluence and treated for 2 hours with 10 ng/ml PMA in the presence or absence of cycloheximide (CHX) as indicated in the Figure. RNA was separated, blotted, and hybridized with probes detecting MADER, c-*fos* and GAPDH, respectively. The basal levels of the three mRNAs are shown in lane 1.

Detailed Description of the Preferred Embodiments:

The practice of the invention will employ, unless otherwise indicated, conventional methods of immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, et al. *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); Maniatis et al. *Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis, a Practical Approach*, Gait, M.J. (ed.), Oxford, England: IRL Press (1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984).

As used in this specification and the appended claims, the singular forms "a," "an" and

"the" include plural references unless the content clearly dictates otherwise.

A. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

An "antigen" includes any substance that may be specifically bound by an antibody molecule. An "immunogen" is a macromolecular antigen that is capable of initiating lymphocyte activation resulting in an antigen-specific immune response. An immunogen therefore includes any molecule which contains one or more epitopes that will stimulate a host's immune system to initiate a secretory, humoral and/or cellular antigen-specific response.

The term "antibody" encompasses polyclonal and monoclonal antibody preparations, as well as preparations including hybrid antibodies, altered antibodies, $F(ab')_2$ fragments, F(ab) fragments, Fv fragments, single domain antibodies, chimeric antibodies, humanized antibodies, and functional fragments thereof which exhibit immunological binding properties of the parent antibody molecule, alone, or linked to another polypeptide, or as a fusion protein.

As used herein, the term "monoclonal antibody" refers to an antibody molecule derived from a single original lymphocyte. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins as well as fragments such as Fab, $F(ab')_2$, Fv, and other fragments derived from a parent monoclonal antibody molecule which fragments exhibit immunological binding properties of the parent molecule.

As used herein, the term "immunological binding" refers to non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and on geometric parameters that equally influence the rate in both directions. Thus, both an "on rate constant" (K_{on}) and an "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. The ratio of K_{off}/K_{on} enables cancellation of all parameters not related to affinity, and is thus equal to the dissociation constant K_d. *See, generally*, Davies et al. (1990) *Annual Rev. Biochem.* 59:439-473.

As used herein, the term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to, or exclude, post expression modifications of the polypeptide; for example, glycosylations, acetylations, phosphorylation and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (e.g., unnatural amino acids), polypeptides with substituted linkages, as well as other modifications known in the

art, both naturally occurring and non-naturally occurring.

The following single-letter amino acid abbreviations are used throughout the text:

Alanine	A	Arginine	R
Asparagine	N	Aspartic acid	D
Cysteine	C	Glutamine	Q
Glutamic acid	E	Glycine	G
Histidine	H	Isoleucine	I
Leucine	L	Lysine	K
Methionine	M	Phenylalanine	F
Proline	P	Serine	S
Threonine	T	Tryptophan	W
Tyrosine	Y	Valine	V

A polypeptide or amino acid sequence "derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 3-5 amino acids, preferably at least 4-7 amino acids, more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence. This terminology also includes a polypeptide expressed from a designated nucleic acid sequence.

As used herein, the terms "oligonucleotide" and "polynucleotide" shall be generic to polydeoxy-ribonucleotides (containing 2-deoxy-D-ribose), to polyribonucleotides (containing D-ribose), to any other type of polynucleotide which is an N-glycoside of a purine or pyrimidine base, and to other polymers containing nonnucleotidic backbones, provided that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA. There is no intended

distinction in length between the term "polynucleotide" and "oligonucleotide," and these terms may be used interchangeably. These terms refer only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, as well as double- and single-stranded RNA and DNA:RNA hybrids, and also include known types of modifications, for example, labels which are known in the art.

By "purified" and "isolated" is meant, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present.

"Homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known in the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when at least about 80%, preferably at least about 90%, and most preferably at least about 95% of the nucleotides or amino acids match over a

defined length of the molecules, as determined using the methods above.

The terms "recombinant DNA molecule," or "recombinant nucleic acid molecule" are used herein to refer to a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature. Thus, the term encompasses "synthetically derived" nucleic acid molecules.

As used herein, the term "cancerous condition" refers to any neoplastic disease characterized by the presence of cancerous cells (or tissue). Cancer cells, unlike benign tumor cells, exhibit the properties of invasion and metastasis and are generally highly anaplastic. The term "cancerous condition," therefore, includes, without limitation, those conditions commonly referred to as melanomas, carcinomas (e.g., carcinomas of the breast, prostate and lung), neuroblastomas, lymphomas and leukemias.

There is a significant overlap in the pattern of mutated genes carried by different cancer cell types, and among individual tumors of the same type. Mutations in the p53 gene, for example, are found in over half of all human tumors. *See, e.g.*, Harris, C.C. (1994) *Science* 262:1980, Harris et al. (1993) *N. Engl. J. Med.* 329:1315, Harper et al. (1992) *Cell* 75:805, Vogelstein et al. (1992) *Cell* 70:523, and Hollstein et al. (1991) *Science* 253:49. As described below, overexpression of the MADER gene has been associated with human malignant melanomas and with breast carcinomas.

A "malignant cell" refers to a cancerous cell which has undergone phenotypic transformation,

such as, but not limited to, transformation by oncogenes, protooncogenes, TS mutations, or other such mechanisms. "Malignant cells" are generally characterized by their capacity for unregulated growth, and have the properties of anaplasia, invasion and metastasis. Such cells have one or more phenotypic derangements which may be expressed as alterations in cellular membranes, in the expression levels of certain cellular proteins (e.g., enzymes involved in nucleic acid synthesis and metabolism), or by the appearance of inappropriate gene products.

The term "melanoma" encompasses those tumors arising from the melanocytic system of the skin and other organs. A "malignant melanoma" is a cutaneous malignant neoplasm of melanocytes, arising *de novo* or from a preexisting benign nevus or lentigo maligna. Malignant melanomas most often occur in the skin.

The terms "analyte" and "nucleic acid analyte" refer to a single- or double-stranded nucleic acid molecule which contains a target nucleotide sequence.

As used herein, the terms "target region" or "target nucleotide sequence" refers to a probe binding region contained within the target molecule. The term "target sequence" refers to a sequence with which a probe will form a stable hybrid under suitable hybridization conditions.

As used herein, the term "probe" refers to a structure comprised of an oligonucleotide, as defined above, which contains a nucleic acid sequence complementary to a nucleic acid sequence present in another molecule of interest. The oligonucleotide regions of probes may be composed of DNA, and/or RNA, and/or synthetic nucleotide analogues.

Two nucleotide sequences are "complementary" to one another when those molecules share base pair organization homology. "Complementary" nucleotide

sequences will combine with specificity to form a stable duplex under appropriate hybridization conditions. Thus, two sequences need not have perfect homology to be "complementary" under the invention, and in most situations two sequences are sufficiently complementary when at least about 85% (preferably at least about 90%, and most preferably at least about 95%) of the nucleotides match over a defined length of the molecule. DNA sequences that are complementary can be identified using Southern blot hybridization under, for example, stringent conditions as defined for that particular system. Southern, E. (1975) *J. Mol. Biol.* 98:503. Defining appropriate hybridization conditions is within the skill of the art. *See, e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989).

As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, dyes, metal ions, ligands (e.g., biotin or haptens) and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range. The term "cofactor" is used broadly herein to include any molecular moiety which participates in an enzymatic reaction. Particular examples of labels which may be used under the invention include fluorescein, rhodamine, dansyl, umbelliferone, Texas red, luminol, NADPH, α-β-galactosidase and horseradish peroxidase.

General Methods:

Central to the present invention is the discovery of a highly conserved gene encoding the approximately 55 kD MADER protein. As described

herein, the overexpression of this gene is associated with human malignant melanomas, and with other types of tumors. cDNA clones encoding MADER were isolated from a human melanoma expression library using cross-reactive monoclonal antibodies produced against the melanoma associated antigen MUC18 (Lehmann et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9891-9895). The nucleotide sequence (SEQ ID NO.: ___), and the predicted amino acid sequence (SEQ ID NO.: ___), of a 2192 bp MADER cDNA molecule are depicted in Figure 1. Two potential translation start sites (ATG), respectively occurring at positions 104 and 119 (where the first site is in a more favorable Kozak consensus sequence (Kozak, M. (1986) *Cell* 44:283-292)), are embedded within an open reading frame encoding a putative protein of 440 amino acids with a deduced molecular weight of approximately 55 kD. A hydrophobicity analysis calculated by the method of Kyte and Doolittle (Kyte et al. (1982) *J. Mol. Biol.* 157:105-132) indicates no evidence of a transmembrane region. The 3′ untranslated region is approximately 770 bp, and has one polyadenylation site (AATAAA) at position 2160. Located within the 3′ region are three ATTTA repeats which have been underlined in Figure 1. ATTTA repeats have been implicated in rapid message turnover (Shaw et al. (1986) *Cell* 46:659-667; Sachs, A.B. (1993) *Cell* 74:413-421). A search of available data banks with the programs FASTA and Blast revealed no significant homology between MADER and known sequences. The above-described cDNA sequence for MADER has been deposited with the EMBL data bank under the accession number X70991 HSDROP9 (GenBank accession number X70991), and is referred to herein as the "Drop9 variant."

The nucleotide sequence (SEQ ID NO.:___) and predicted amino acid sequence (SEQ ID NO.:___) of a shorter splice variant of MADER are depicted in Figure

2. The shorter splice variant is referred to herein as the "Drop8 variant." Drop8 lacks 192 base pairs (which are italicized and underlined in Figure 2) from the Drop9 variant sequence, and is presumably a splice variant of the longer Drop9 sequence, lacking one exon. Monoclonal antibodies produced against the Drop9 MADER variant also bind to the Drop8 variant. These monoclonal antibody molecules are described below.

A longer version of MADER was obtained from human placental cDNA by PCR amplification using the following primers derived from the Drop9 variant sequence: GCCAACCTCCTTTCCTACTATG (SEQ ID NO.:___) and GGCGAAGCTTCTGCCGGCTGGCCTCAGCCTC (SEQ ID NO.:___). The resulting fragment was combined with the 5' end of the human cDNA obtained by amplification of cDNA ends, and cloned into a cytomegalovirus-driven expression vector to yield a construct termed pCMVNAB2. Svaren et al. (1996) *Molec. Cell. Biol.* 16:3545-3553. A 1735 bp nucleotide sequence (mRNA) (SEQ ID NO.:___), and a full-length, 525 residue amino acid sequence (SEQ ID NO.:___) of the longer MADER variant are depicted in Figure 3. Residues 50-525 of the longer MADER amino acid sequence (Figure 3) are identical to residues 1-475 of the Drop9 variant (Figure 1). Thus the longer sequence merely includes an additional 49 N-terminal amino acids. The mRNA sequence of this longer MADER variant has been deposited with GenBank under accession number U48361. This longer version of MADER has been reported to bind to erg-1 (also known as NGFI-A), and inhibit its activity. Svaren et al. (1996), *supra*.

A 320 residue amino acid sequence (SEQ ID NO.:___) of yet a further MADER polypeptide is also shown in Figure 3. This particular splice variant is approximately one-third the size of the Drop9 variant, and has been observed in human placental tissue.

Svaren et al. (1996) *Molec. Cell. Biol.* 16:3545-3553. Analysis of DNA encoding this shorter splice variant has revealed that it has a deleted internal sequence, producing a frameshift that causes premature termination of translation. Termination results in the loss of approximately one-third of the full-length, 525 amino acid MADER molecule. Analysis of human placental DNA has revealed that this alternatively spliced form of MADER is found in an approximately 1:1 ratio with the full-length molecule. Further, the shorter, 320 amino acid MADER polypeptide has an altered function, and fails to repress erg-1 activity. Svaren (1996), *supra*.

Referring in particular to Figure 1, the putative 55 kD MADER protein is rich in proline residues which are concentrated in two regions, one near the N-terminus and one near the C-terminus. The N-terminal region lies between amino acids 61-185, and contains a high proportion of glycine (23%), and proline (20%). The second domain, occurring between amino acids 371 and 458, is rich in proline (20%) and leucine (16%). Both regions contain repeated S(T)PXX motifs (indicated by boxed residues in Figure 1) which are characteristic of gene regulatory DNA binding proteins (Suzuki, M. (1989) *J. Mol. Biol.* 207:61-84). Specifically, six Ser-Pro-Xaa-Xaa (SPXX) motifs, and one Thr-Pro-Xaa-Xaa (TPXX) motif, are located within the N-terminal proline-rich region, while the C-terminal domain includes one TPXX and one SPXX motif. The fact that the two proline-rich domains of MADER contain repeats of the sequence motif S(T)PXX is consistent with a role for MADER in the regulation of gene expression, since the presence of such repeats has been found to be common to all types of regulatory DNA binding proteins including steroid receptors, the helix-turn-helix and zinc finger families, polymerases, and H1 histones.

The predicted MADER protein also contains a putative bipartite nuclear localization signal, occurring in the middle of the coding region (indicated by boxed residues and an overlying dotted line in Figure 1). A comparison between the MADER signal sequence and that of nucleoplasmin (Robbins et al. (1991) *Cell* 64:615-623; Dingwell et al. (1991) *TIBS* 16:478-481) reveals a strong sequence motif homology. Further, the putative MADER amino acid sequence includes three CK-2 sites within 30 residues of the nuclear localization signal. Flanking CK-2 phosphorylation sites have been found to be important in the regulation of nuclear localization for some proteins (Rihs et al. (1991) *EMBO J.* 10:633-639). These features suggest that the MADER protein is targeted to the nucleus, and immunohistochemical analyses using MADER-specific monoclonal antibodies in cell lines and human tissue samples indicate that the MADER protein is in fact a nuclear protein. Studies with murine cell lines have also demonstrated nuclear localization of MADER. Svaren et al. (1996) *Molec. Cell. Biol.* 16:3545-3553.

As described in detail below, MADER mRNA is detectable at low levels in a number of cell lines derived from various tumors. The expression of MADER in such cell lines can be rapidly up-regulated in response to growth factors or mitogens. Such increased expression is transient, generally returning to basal levels within about six hours. Expression of detectable levels of MADER protein in normal cell lines and frozen tissue is relatively rare. However, strong uniform nuclear expression of MADER has been observed in all malignant melanoma lesions thus far examined. Since such expression has not been seen in normal epidermal melanocytes and benign melanocytic tumors (nevi), it appears that malignant transformation of melanocytes is associated with

over-expression of the MADER protein. Further, over-expression of the MADER protein may be associated with other malignant conditions.

Accordingly, the MADER protein is used in the practice of the invention to provide a malignant melanoma-associated marker molecule for immunohistochemical diagnosis of melanocytic lesions. Particularly, anti-MADER monoclonal antibodies are used in various diagnostic methods to detect melanoma malignancies in tissue samples. Anti-MADER monoclonal antibodies are also used to detect other cancers, such a breast carcinomas, in tissue samples. The MADER protein, and fragments or analogs thereof, also find use herein as immunogens for eliciting an immune response against cells which over-express MADER in an immunized subject.

Fluorescence *in situ* hybridization (FISH) localization of the MADER gene to human chromosomes has been carried out in order to gain further insight into the function of MADER. Svaren et al. (1996), *supra*. In both metaphases and prometaphases, distinct banding patterns in individual chromosomes permitted direct localization of MADER to band 12q13.3-14.1. This region is frequently rearranged in several solid tumors, lipomas, liposarcomas, gliomas, and adenomas of salivary glands. Reifenberger et al. (1995) *Cancer Res.* 55:731-734; Schoenberg et al. (1995) *Genomics* 26:265-271; Solomon et al. (1991) *Science* 254:1153-1160; Van deVen (1995) *Genes Chromosomes Cancer* 12:296-303. Region 12q13-15 is also known as a preferential site for human papillomavirus integration in cervical carcinomas (Popescu et al. (1987) *J. Virol.* 61:1682-1685; and Sastre-Garau et al. (1990) *Cancer Genet. Cytogenet.* 44:253-261), and microcell fusion-mediated transfer of the particular region of chromosome 12 into a prostate cancer cell line can

suppress tumorigenicity (Berube et al. (1994) *Cancer Res.* 54:3077-3081).

The MADER gene can thus be used as a target to detect translocation events in tumors, where translocation of the MADER gene gives rise to over-expression of MADER in malignant cells. Further, MADER RNAs are useful as targets in methods of determining the presence or absence of MADER over-expression in a tissue sample, where such over-expression is indicative of the presence of malignant cells, including malignant melanoma cells.

MADER polynucleotides also find use herein as targets in various therapeutic methods for treating malignant melanoma, and other cancers, in a subject. Compositions including MADER DNA polynucleotide immunogens are used to elicit an immune response against cells which over-express MADER in an immunized subject. Further, MADER mRNAs are used as targets for antisense oligonucleotides in antisense therapies directed at inhibiting the expression of MADER in a treated subject. MADER RNAs are also used as targets for ribozymes capable of degrading MADER mRNAs.

Thus, one embodiment of the present invention pertains to the production of monoclonal antibody molecules capable of specifically binding to the approximately 55 kD MADER protein. Anti-MADER monoclonals can be readily produced by one skilled in the art using general hybridoma methodology. In this regard, immortal antibody-producing cell lines can be created by cell fusion or by other techniques such as direct transformation of B lymphocytes with oncogenic DNA or by transfection with Epstein-Barr virus. *See, e.g.*, M. Schreier *et al.*, *Hybridoma Techniques* (1980); Hammerling *et al.*, *Monoclonal Antibodies and T-cell Hybridomas* (1981); Kennett *et al.*, *Monoclonal Antibodies* (1980).

More particularly, monoclonal antibodies can be readily produced using the method of Kohler and Milstein, *Nature* (1975) 256:495-497, or a modification thereof. Typically, a suitable murine host animal is immunized with one or more MADER peptide antigens. In order to enhance immunogenicity, the peptide antigens can be linked to a carrier prior to immunization. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Furthermore, the MADER antigen may be conjugated to a carrier molecule in order to enhance the immunogenicity thereof.

After immunization, the animals are sacrificed and the spleen is obtained and dissociated into single cells. Dissociated spleen cells, or isolated B-lymphoblasts (specific, or specific and non-specific) are then induced to fuse with myeloma cells to form hybridomas which are then cultured in a selective medium (*e.g.*, hypoxanthine, aminopterin, thymidine medium, "HAT"). Available murine myeloma lines, such as those available from the American Type Culture Collection (ATCC, Rockville, MD) can be used in the fusions. The resulting hybridomas are plated by limiting dilution, and assayed for the production of antibody molecules which bind specifically to the immunizing MADER antigen. The selected monoclonal antibody-secreting hybridomas are then cultured, either *in vitro* (e.g., in tissue culture bottles or hollow fiber reactors), or *in vivo* (e.g., as ascites in mice).

Suitable MADER antigens used in the production of the above monoclonal antibodies include

immunogenic peptide fragments that are at least about 5 amino acids in length, preferably 7-10 amino acids in length, and most preferably at least about 10 to 15 amino acids in length. There is no critical upper limit to the length of the fragment, which can comprise an entire MADER sequence, or alternatively one or more MADER peptides fused to a heterologous protein sequence.

The identification of suitable MADER epitopes is readily accomplished using techniques well known in the art. *See, e.g.*, Geysen et al. *Proc. Natl. Acad. Sci. USA* (1984) 81:3998-4002 (general method of rapidly synthesizing peptides to determine the location of immunogenic epitopes in a given antigen); U.S. Patent No. 4,708,871 (procedures for identifying and chemically synthesizing epitopes of antigens); and Geysen et al., *Molecular Immunology* (1986) 23:709-715 (technique for identifying peptides with high affinity for a given antibody).

Predicted MADER amino acid sequences (SEQ ID NOs.: ___, ___, ___, and ___) are depicted in Figures 1-3. Thus, MADER peptide antigens may be synthesized by protein synthesis techniques known to those of skill in the art. In general, these methods employ either solid or solution phase synthesis methods. *See, e.g.*, J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, 2nd Ed., Pierce Chemical Co., Rockford, IL (1984) and G. Barany and R. B. Merrifield, *The Peptides: Analysis, Synthesis, Biology*, editors E. Gross and J. Meienhofer, Vol. 2, Academic Press, New York, (1980), pp. 3-254, for solid phase peptide synthesis techniques; and M. Bodansky, *Principles of Peptide Synthesis*, Springer-Verlag, Berlin (1984) and E. Gross and J. Meienhofer, Eds., *The Peptides: Analysis, Synthesis, Biology, supra*, Vol. 1, for classical solution synthesis.

The peptides can also be produced using recombinant techniques that are known in the art. For example, a DNA sequence encoding all or a portion of a MADER peptide can be synthesized using standard methods. *See, e.g.*, Edge (1981) *Nature* 292:756; Nambair *et al.* (1984) *Science* 223:1299; Jay *et al.* (1984) *J. Biol. Chem.* 259:6311. Alternatively, the sequence can be derived from genomic or cDNA. The DNA is cloned into an appropriate expression vector, either procaryotic or eucaryotic, using conventional methods. Suitable host cells are then transfected with the expression vector and cultured under conditions allowing for expression of the MADER peptides. Alternatively, MADER peptide antigens can be produced by enzymatic or chemical cleavage of purified MADER protein. Such procedures are conventional and well-known in the art.

Once produced, the anti-MADER monoclonal antibodies are available for use in immunohistochemical methods of detecting malignant melanoma cells, or other cancerous cells, present in a tissue sample. Functional fragments of the anti-MADER antibody molecules will also find use with the present invention, and can be produced by cleaving a constant region from an antibody molecule using, *e.g.*, pepsin, to produce $F(ab')_2$ fragments. Alternatively, Fab fragments can be produced using known methods, *e.g.*, by digestion of the monoclonal antibodies with papain.

Tissue samples suspected of containing malignant melanoma cells, or other cancerous cells, are then exposed to the anti-MADER monoclonal preparations, and the presence or absence of bound antibodies on the tissue sample is determined as an indication of the presence or absence of a malignancy. The tissue samples generally comprise nodular or tumorous skin lesions that have been biopsied for histological analysis. The samples are prepared for

analysis using known techniques. For example, biopsy tissue samples may be reduced to single cell suspensions using techniques known in the art such as physical maceration, sonication, centrifugation or the like. Cell lysates are then obtained which are suitable for reaction with the monoclonal antibody molecules.

More preferably, tissue sections are prepared using either freezing or paraffin sectioning methods. Paraffin tissue sections (e.g., 5 μm sections) can be prepared and deparaffinized in xylene, hydratred through graded alcohols and placed on a suitable substrate such as a microscope slide. Frozen tissue sections can be prepared by immediately deep freezing a 2-3 mm tissue slice (obtained from a fresh surgical biopsy) in OCT compound using the method of Ruiter, D.J., (1990) *Curr. Opinion Oncol.* 2:377-387. The tissue sections can then be fixed in acetone and placed on a slide. The prepared slides are then incubated with a preparation containing one or more of the above-described anti-MADER monoclonal antibodies.

In order to visualize positive antibody-antigen binding events, the primary anti-MADER monoclonal antibody (in a direct procedure), or a second or third antibody capable of recognizing the primary antibody (in an indirect procedure) can be labeled with a suitable detectable moiety. Suitable labels include, for example, enzymes, radioisotopes, fluorescers, chromophores, chemiluminescers, dyes, metal ions or ligands which provide for rapid and sensitive detection using techniques known in the art. Particularly suitable labels include enzymes such as horse radish peroxidase or alkaline phosphatase, or complexes such as avidin-biotin. Depending on the nature of the label, a number of techniques to detect the presence of the label are known, e.g.,

fluorometric, spectrophotometric, autoradiography, scintillation counting, and visual (e.g., colorimetric or chemiluminescence) techniques.

In another embodiment of the invention, methods are provided for detecting the presence of cells that over-express the MADER gene. As described above, over-expression of MADER has been correlated with malignant melanoma. Accordingly, oligonucleotide probes are provided herein, comprising a nucleotide sequence having at least a 17 base region that is complementary to a region of mRNA transcribed from the MADER gene. The oligonucleotide probes may be composed of DNA, RNA, and/or synthetic nucleotide analogues. If a natural MADER nucleic acid sequence is used, the nucleotide may be isolated from a suitable biological source using known methods such as by the chemical action of detergents, bases, acids, chaotropic salts or mixtures thereof. If desired, the average size of the nucleic acid sequence may be decreased by enzymatic, physical or chemical means, e.g., using restriction enzymes, sonication, chemical degradation and the like.

Alternatively, the oligonucleotide probes may be synthetically derived, using a combination of solid phase direct oligonucleotide synthesis chemistry and enzymatic ligation methods which are conventional in the art. cDNA sequences of the MADER gene are known, and are depicted in Figure 1 (SEQ ID NO.: __) and Figure 2 (SEQ ID NO.: __). A MADER mRNA sequence is also readily available (GenBank accession number U48361), and is depicted in Figure 3 (SEQ ID NO.: __). Synthetic sequences may be prepared under the invention using commercially available oligonucleotide synthesis devices such as those devices available from Applied Biosystems, Inc. (Foster City, CA).

The oligonucleotide probes are labeled using a suitable detectable moiety. A wide variety of

methods of detectably labeling target oligonucleotides are known in the art. *See, e.g.*, Dunn et al. (1980) *Methods Enzymol.* 65:468-478; Palva et al. (1983) *Journal Clin. Micro.* 18:92-100; Ranki et al (1983) *Gene* 21:77-85; Polsky-Cynkin et al. (1985) *Clin. Chem.* 31:1438-1443; and U.S. Patent Nos. 4,486,539 and 4,563,419.

Once prepared, the labeled probes are used in mixed phase hybridization assays to detect the presence of MADER mRNAs in a selected tissue sample. A number of suitable mixed-phase hybridization techniques are well known in the art. Proceeding with the method, tissue samples suspected of containing cells which over-express the MADER gene are reduced to single cell suspensions using known techniques. The cell suspensions are treated, e.g., lysed, to release the cellular oligonucleotides including RNAs. Chemical lysing may be performed using dilute aqueous alkali, e.g., 0.1 to 1.0 M sodium hydroxide. The alkali serves to denature the RNAs. Alternative methods of denaturation and cell lysing are known in the art and may employ, among other things, elevated temperature, organic reagents (e.g., alcohols, amides, ureas, phenols and sulfoxides), inorganic ions (chaotropic salts such as sodium trifluoroacetate, sodium trichloroacetate, sodium perchlorate, guanidinium isothiocyanate, sodium iodide, potassium iodide, sodium isothiocyanate and potassium isothiocyanate) and combinations thereof.

The released RNA is extracted from the lysate and purified using methods such as density gradient centrifugation, ethanol precipitation, phenol extraction, or the like. *See, e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989). Additionally, the RNA may be digested using restriction endonucleases to provide smaller nucleotide segments. The sample RNAs are

immobilized to a solid support or substrate. Immobilization of oligonucleotides to a suitable substrate may be performed using conventional techniques. *See, e.g.*, Letsinger et al. (1975) *Nucl. Acids Res.* 2:773-786; "*Oligonucleotide Synthesis, a Practical Approach*," Gait, M.J. (ed.), Oxford, England: IRL Press (1984). Commonly used solid supports include nitrocellulose or nylon, and methods of immobilizing nucleotides to such supports include transfer of selected sequences onto nitrocellulose filters or nylon membranes using Southern blot, colony and plaque blot, or dot and slot blot techniques. Leary et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:4045-4049; Meinkoth et al. (1984) *Anal. Biochem.* 138:267-284.

The immobilized cellular RNA and the detectably labeled oligonucleotide probes are incubated under hybridizing conditions to provide an RNA-probe hybrid. Hybridization generally takes from about 30 minutes to about 2 hours. The hybridization occurs at the highest rate at approximately 25°C below the temperature at which the nucleotide hybrid is 50% melted (the "T_m"). The T_m for a particular hybridization pair will vary with the length and nature of the nucleotides and may be readily determined by those of ordinary skill in the art.

In general, hybridizations are carried out in a buffered aqueous medium typically formulated with a salt buffer, detergents, nuclease inhibitors and chelating agents. Such formulations may be selected to preclude significant non-specific binding of nucleotides with the support surface. Depending on the nature of the particular oligonucleotide binding pair, various solvents may be added to the medium such as formamide, dimethylformamide and dimethylsulfoxide, and the stringency of the hybridization medium may be controlled by temperature, pH, salt concentration,

solvent system, or the like. Defining appropriate hybridization conditions is within the skill of the art. *See, e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989).

After the subject oligonucleotides have been incubated under suitable hybridization conditions for a sufficient time to allow formation of duplexes, a washing step is performed to provide an immobilized RNA-probe complex substantially free of unbound probe. A detection step is then carried out to detect the presence of any labeled probe on the substrate. In this manner, a signal can be obtained which is indicative of the presence or absence of cells in the tissue sample which over-express the MADER gene. The detection step is carried out under suitable conditions, such as in a detection solution formulated according to a particular detection means (e.g., where the label employed is an enzyme, the solution is formulated to include the selected enzyme substrate and any necessary reagents).

In a related embodiment of the invention, *in-situ* hybridization is carried out to detect the presence of MADER mRNAs in a selected tissue sample. A number of suitable *in-situ* hybridization methods are generally known in the art. For example, tissue samples can be post-fixed, frozen, and then sectioned to provide 5 to 35 μm sections. The sections can be mounted onto suitable substrates, such as polylysine-coated slides, and treated with proteinase K, aceticanhydride, and then dehydrated in graded alcohol. The mounted sections can then be incubated under hybridizing conditions with detectably labeled oligonucleotide probes, prepared as described above, to provide an RNA-probe hybrid. The hybridizations are carried out as above, the mounted washed, and then

a detection step is performed to detect the presence of any labeled probe on the substrate.

In yet another embodiment of the invention, a method is provided for detecting a melanoma malignancy, or other cancerous condition, in a tissue sample, which method generally comprises visualizing the presence or absence of a translocation of MADER in cellular genomes using fluorescence *in situ* hybridization (FISH). Such techniques are generally known in the art. *See, e.g.*, Polak et al., Eds. (1990) *In Situ Hybridisation—Principals and Practice*, Oxford University Press, Oxford; Wilkinson, D.G., Ed. (1992) *In Situ Hybridization: A Practical Approach*, Oxford University Press, Oxford; Gray et al. (1992) *Cancer* 69:1536-1542; Trask, B.J. (1991) *Trends Genet.* 7:149-154. The method of the invention generally entails providing immobilized chromosomal target DNA that has been obtained from a tissue sample suspected of including cells having undergone a MADER translocation. The target DNA is immobilized to a suitable substrate (e.g., fixed to a microscope slide) using oligonucleotide immobilization techniques such as those described above.

Oligonucleotide probes, having a nucleotide sequence that is complementary to a MADER nucleotide sequence, are provided as described above. In the practice of the invention, the probes are generally synthetically derived using a combination of conventional solid phase direct oligonucleotide synthesis chemistry and enzymatic ligation methods. "*Oligonucleotide Synthesis, a Practical Approach*," Gait, M.J. (ed.), Oxford, England: IRL Press (1984). Further, the oligonucleotide probes are preferably DNA fragments ranging from about 200 to 1000 bp in length which have been labeled with a fluorescent moiety. However, the length of the probe is not considered limiting in the present invention. Suitable

fluorescent labels include, but are not limited to, fluorescein isothiocyanate (Fitc), phycoerythrin (PE), rhodamine and Texas red.

Both the labeled oligonucleotide probe and the immobilized target chromosomal DNA are denatured separately, then incubated together under hybridizing conditions to provide a target-probe hybrid. After any unbound probe is washed off, the bound probe is detected using standard fluorescent microscopy. In this manner, the position of the MADER gene sequence can be readily visualized to determine the presence or absence of MADER translocation events.

In yet another embodiment of the invention, novel compositions containing MADER immunogens are provided for use in immunotherapeutic methods for treating a subject having malignant melanoma, or another cancerous condition. MADER immunogens can be obtained from either MADER polypeptides or polynucleotides. Polypeptide MADER immunogens can comprise a natural or synthetically derived full length protein, or one or more immunogenic fragments of a MADER polypeptide molecule. Immunogenic MADER peptide fragments comprise a MADER amino acid sequence of at least about 6 residues or greater. Polynucleotide MADER immunogens can comprise one or more DNA molecules containing polynucleotide sequences of at least about 18 to 30 bp that are substantially homologous to regions of the MADER gene. MADER polynucleotide and polypeptide sequences of various lengths have been described herein, and are depicted in Figures 1-3.

The MADER immunogens are generally combined with a pharmaceutically acceptable vehicle to provide compositions for use in eliciting an immune response against cells over-expressing MADER in an immunized subject. As used herein, "a pharmaceutically acceptable carrier or vehicle" includes any and all

solvents, dispersion media, antibacterial and antifungal agents that are substantially non-toxic to humans. The use of such media and agents for pharmaceutically active substances is well known in the art. Particularly, the compositions may be emulsified or the active ingredient encapsulated in liposome vehicles. The MADER immunogen can be mixed with vehicles containing excipients which are pharmaceutically acceptable and compatible with the immunogen. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and/or pH buffering agents. Actual methods of preparing such compositions are known, or will be apparent, to those skilled in the art. *See, e.g., Remington's Pharmaceutical Sciences*, Mack Publishing Company, Easton, Pennsylvania, 18th edition, 1990. The compositions of the present invention will, in any event, contain a quantity of the MADER immunogen adequate to achieve the desired immunized state in the subject being treated.

Further, suitable adjuvants may be incorporated into the compositions to enhance the immunogenicity of the compositions. A number of suitable adjuvants are known in the art, including organic molecules (e.g., muramyl dipeptide and tuftsin), synthetic adjuvants (e.g. levamisole and isoprinosine) and other agents such as aliphatic nitrogenous bases (e.g., dimethyldioctadecylammonium bromide (DDA) and N,N-dioctadecyl-N,N-bis(2-hydroxyethyl)propanediamine ("avridine"). Such agents act non-specifically to enhance the immunogenicity of a particular composition, thus reducing the quantity of antigen necessary in any given vaccine, as well as

the frequency of injection. A.C. Allison (1979) *J. Reticuloendothel. Soc.* 26:619-630.

The MADER immunogen compositions can be administered to a subject using parenteral (e.g., intravenous or intramuscular) injection, or by other suitable routes such as subcutaneous or intradermal injection, transdermal delivery, or like modes of administration. Effective dosages for the MADER immunogens in immunotherapies can be determined by routine experimentation, keeping in mind the objective of the treatment. Basically, pharmaceutical forms of MADER immunogens suitable for injectable use include sterile aqueous solutions or dispersions. Sterile injectable solutions can be prepared by incorporating the MADER immunogens of the invention into an appropriate solvent, such as sodium phosphate-buffered saline, followed by filter sterilization.

In one particular immunotherapeutic method, MADER proteins, or MADER peptide fragments, are coupled to approximately 10-35 μm biodegradable beads using known methods of attachment. DeLuca et al. (1987) "*Porous Biodegradable Microspheres for Parenteral Administration*," Topics in Pharmaceutical Sciences, Elsevier Science Publishers, B.V., Amsterdam. Immunization with such particulate antigens targets the proteins to the major histocompatibility complex class I pathway, thus eliciting a cytotoxic response. Kovacsovics-Bankowski et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:4942; Kovacsovics-Bankowski et al. (1995) *Science* 267:243. Individuals are primed and boosted several times at appropriate intervals, e.g., at 4 week intervals, until a cytotoxic response is measurable using a standardized *in vitro* chromium release assay (with the individual's own peripheral blood cells as effector cells and the individual's tumor cells as the target cells).

In yet a further therapeutic application of the invention, MADER antisense molecules are used in tumor therapies. A number of pharmaceuticals have been described in the art which are capable of binding specifically and predictably to certain nucleic acid target sequences in order to inhibit or modulate the expression of disease-causing genes. Neckers et al (1992) *Crit. Rev. Oncogenesis* 3:175; Simons et al. (1992) *Nature* 359:67; Bayever et al. (1992) *Antisense Res. Dev.* 2:109; Whitesell et al. (1991) *Antisense Res. Dev.* 1:343; Cook et al. (1991) *Anti-Cancer Drug Design* 6:585; Eguchi et al. (1991) *Annu. Rev. Biochem.* 60:631; Uhlmann et al. (1990) *Chem. Rev.* 90:543. Accordingly, antisense oligonucleotides capable of selectively binding to target MADER sequences are provided herein for use in antisense therapeutics. The antisense oligonucleotides are synthetic oligonucleotides that bind via Watson-Crick base pairing to complementary regions of MADER mRNA, thereby inhibiting MADER protein biosynthesis.

In the practice of the invention, synthetic MADER antisense oligonucleotide molecules can be prepared using solid phase chemistry techniques in combination with the phosphoramidite method. *See, e.g.*, Beaucage et al. (1992) *Tetrahedron* 48:2223. However, any suitable synthetic method known in the art may be used herein to provide the subject antisense oligonucleotides. The molecules range from about 10 to about 30 bases in length, wherein a 17-mer oligonucleotide sequence is most preferable since such a sequence is generally regarded to appear statistically only once in the human genome. The antisense oligonucleotides must be sufficiently stable in serum and within target cells, must be capable of penetrating cell membranes and of forming stable complexes with their target MADER sequences under physiological conditions. Accordingly, a number of

chemical modifications to the "natural" structure of the synthetic oligonucleotides need to be made such as, but not limited to, modifications or replacement of the phosphodiester backbone and base and sugar modifications. Such modifications are within the general skill of the art.

Once constructed, the MADER oligonucleotide antisense molecules are combined with suitable pharmaceutically acceptable vehicles to provide compositions for administration (e.g., via parenteral injection) to a subject. Improved cellular uptake of the antisense molecules can be achieved by lipophilic derivatization of the oligonucleotides (e.g., as lipophilic conjugates at the 5′ terminus), by conjugation to poly-L-lysine, or by packaging into antibody-targeted liposomes. The subject compositions can preferably be injected directly into malignant melanoma tissue, or into other cancerous tissue. A construct containing the MADER gene is also suitable for DNA immunizations using known techniques. *See, e.g.*, U.S. Patent No. 5,589,466 to Felgner et al.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure of the invention. The examples are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some experimental error and deviation should, of course, be allowed for.

EXAMPLES

Reagents and Antibodies

Unless otherwise specified, all chemicals were purchased from Sigma Chemical Company (St. Louis MO). Phytohemagglutinin-M (PHA) was obtained from DIFCO Laboratories (Detroit MI). An interspecies DNA blot (Zoo blot) was purchased from Clontech (Palo Alto, CA) and contained *Eco*RI digested DNA isolated from human placenta, rat kidney, dog kidney, rabbit kidney, Rhesus kidney, bovine kidney, chicken liver and Saccharomyces cerevisiae.

The monoclonal antibodies 5B3 (IgG1) and 5H1 (IgG1) were produced against recombinant MADER protein as described above. The monoclonal antibody W6/32 (IgG2a) directed against HLA class I molecules was obtained from the American Type Culture Collection (Rockville MD). Monoclonal antibody M701 (IgG1) directed against the CD45 leukocyte antigen was obtained from Dako (Glostrup, DK), and monoclonal antibody G7A5 (IgG1) directed against the melanoma-nevus associated proteoglycan (Garrigues et al. (1986) *J. Cell. Biol.* 103:1699-1710) was obtained from Immunotech (Marseille, FR).

Cells and Tissue

Cell lines were either obtained from the American Type Culture Collection (ATCC, Rockville, MD) or established *de novo* for the experiment. The cells were routinely grown in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS). Peripheral blood mononuclear cells (PBMC) from normal volunteers were isolated from heparinized whole blood by density centrifugation on Ficoll (density 1.077). Tissue samples were snap frozen in liquid N_2 shortly after removal and stored at -80°C until use.

RNA Isolation, Northern and Southern Blot Analysis

Total RNA was prepared from cells and tissues using a modified method of Okayama et al. (1987) *Methods Enzymol.* 154:3-28. Cesium trifluoroacetate (CsTFA, Pharmacia) was used for isolation and separation of RNA by isopycnic centrifugation. 20 μg total RNA were denatured in formaldehyde, separated on a 1.2 % agarose/formaldehyde gel, transferred to Hybond-N nylon membrane (Amersham, Braunschweig), and UV cross-linked. Genomic DNA was digested with restriction endonucleases, electrophoresed, transferred to Hybond-N nylon membrane and hybridized as described for plasmid blots. Northern blots hybridized with MADER probes were washed only down to 0.3x SSC/0.1%SDS. The oligonucleotides detecting c-*fos* (5'—CCCCGGCCGGGGACGCGCTGCTGCAGCGGGAGGA TGACGCCTCGTAGTCTGCG—3') (SEQ ID NO.: ___) and GAPDH (glycerin aldehyde-3 phosphate dehydrogenase, exon 1: 5'—CCCTGGTGACCAGGCGGCCAATACGGCCAATCCGTTGACTCCGAC TTTCCAC—3') (SEQ ID NO.: ___) (Dugaiczyk et al. (1985) *Biochem.* 22:1605-1613), were end-labeled using terminal desoxynucleotidyl transferase (Gibco BRL). Filters were hybridized and washed as previously described (Sers et al. (1993) *Proc. Natl. Acad. Sci USA* 90:8514-8518) at 65°C. Quantitation of autoradiograms was performed with an Elscript densitometer (Hirschmann, Unterhaching).

Example I
cDNA Library Screening and DNA Sequencing of MADER

Screening of the Mel JuSo cDNA expression library in lambda gt11 was carried out using the monoclonal antibodies MUC18BA.1, MUC18BA.2 and MUC18BA.3 as previously described (Lehmann et al.

(1989) *Proc. Natl. Acad. Sci. USA* 86:9891-9895). An additional $3x10^6$ independent clones derived from this library, a second human melanoma cDNA library in the lambda gt11 vector (Clontech), a melanoma cDNA library in the Uni-ZAP XR vector obtained from Stratagene (La Jolla, CA), and a testis cDNA library in lambda gt10 (Clontech) were each screened with 241 bp *Eco*RI/ *Bgl*II 5' MADER cDNA fragment to obtain longer cDNA clones. The probes were labeled with [($-^{32}$P] dATP (Amersham, UK) using random priming. Membranes were hybridized overnight at 65°C in 6x SSC/5x Denhardt's solution/0.5 % SDS/salmon sperm DNA (20 μg/ml). The filters were washed at 65°C in 3x SSC /0.1% SDS, 1x SSC/0.1% SDS, 0.3x SSC/0.1% SDS, and 0.1x SSC/0.1% SDS (40 minutes each).

Double-stranded DNA sequencing reaction was performed on the various clones in pUC18 using the dideoxy nucleotide chain termination method (Sanger et al. (1977) *Proc. Natl. Acad. Sci. USA* 74:5436-5467) with Sequenase enzyme (USB, Amersham) and with an AutoRead Sequencing Kit (Pharmacia, Uppsala) using oligonucleotides complementary to the vector and to the cDNA insert.

Example II
Southern Blot Analysis of MADER

Genomic Southern blot analysis was performed with DNA from normal individuals and from a variety of tumor cell lines. Referring now to Figure 4, genomic DNA from EBV-transformed B cells obtained from a normal individual was digested with the restriction endonucleases *Bam*HI and *Hind*III and gel electrophoresed. The positions of the molecular weight markers are indicated on the left of the gel. As can be seen in Figure 4, single MADER bands of 10.5 kb and 7.5 kb were obtained with the *Bam*HI and *Hind*III

digestions. This is consistent with a single copy gene. Analysis of DNA from 28 unrelated normal individuals with *Bam*HI, *Pst*I and *Eco*RI provided no evidence for restriction fragment length polymorphism (RFLP).

Hybridization of human cDNA with DNA isolated from a variety of different species indicates that MADER is highly conserved. Referring now to Figure 5, a Genomic Zoo blot is shown containing *Eco*RI digested DNA from human (lane 1), Rhesus monkey (lane 2), rat (lane 3), mouse (lane 4), dog (lane 5), cow (lane 6), rabbit (lane 7), chicken (lane 8), and *S. cerevisiae* (lane 9). Arrowheads in the Figure indicate the location of the weakly hybridizing bands in the rat (lane 3) and the chicken (lane 8). The blots were hybridized with ^{32}P-labeled complete MADER cDNA. Cross-hybridization under stringent conditions was observed with all species examined, including *Saccharomyces cerevisiae* (lane 9). The discrepancy observed between the signal intensity with the rat (lane 3) and mouse (lane 4) DNAs was due to the low amount of rat DNA on the blot (as was evident from the photograph of the ethidium bromide stained gel). The equivalent signal intensities observed with the human (lane 1) and yeast (lane 9) DNAs was due to the much smaller size of the yeast genome and the fact that equal amounts of DNA were applied to the gel.

Example III

Induction of MADER Expression

In order to determine if MADER mRNA expression is induced by mitogens and growth factors, the following experiments were carried out. For stimulation with phorbol 12-myristate 13-acetate (PMA) or serum, cells from the melanoma cell line Mel JuSo were seeded into multiple bottles and grown to 80%

confluence. FCS or PMA was added directly to the medium at the appropriate concentration. Serum was used at a final concentration of 20%, while PMA was freshly prepared from a stock solution of 1 μg/ml in acetone and used at a final concentration of 10 ng/ml. At various times following exposure to these reagents, the cells were harvested and total cellular RNA was isolated. For mitogen stimulation of PBMC, Mel JuSo cells were cultured at a concentration of 1×10^6 per ml in normal culture medium and PHA added at a 1:100 dilution.

The kinetics of serum or PMA induced MADER mRNA expression were compared with that of c-*fos*, a classical immediate early response gene. Mel JuSo cells exposed to FCS or PMA were harvested at 0.5, 1, 2, 4, and 6 hours following stimulation in order to assay for MADER mRNA induction. After lysis, 20 μg total RNA was separated from the harvested cells as previously described. GADPH was used as a control for RNA loading. Blots were hybridized with [^{32}P]-labeled complete MADER cDNA, or with labeled oligonucleotides detecting c-*fos* or GAPDH. All blots were analyzed by autoradiography and densitometry. The results are depicted in Figure 6.

As can be seen in Figure 6, exposure of the Mel JuSo cells to serum or phorbol ester (phorbol 12-myristate 13-acetate, PMA) led to an increase in MADER expression which reached a maximum by 2 hours. By approximately 4 hours, expression had decreased by more than 50%, and expression reached basal levels by approximately 6 hours. Densitometric measurements indicate an increase in MADER mRNA of approximately 10x after stimulation. In contrast to MADER, c-*fos* expression peaked at 30 minutes and was undetectable by 2 hours.

The ability to up-regulate MADER expression in normal leukocytes stimulated with the PHA mitogen

was ascertained as follows. Freshly isolated PBMC were exposed to PHA, and cells were harvested at 1, 2, 4, 6, and 10 hours after exposure. RNA isolation and Northern analysis were performed as described above. GADPH was used as a control for RNA loading. All blots were analyzed by autoradiography and densitometry. The results are depicted in Figure 7. As can be seen, MADER expression in normal leukocytes can also be up-regulated in response to mitogen exposure. The kinetics of mitogen induced MADER expression in normal lymphocytes resembles that observed in tumor cell lines following serum or phorbol ester stimulation, reaching a peak at 2 hours and returning to basal levels by 6 hours.

In order to ascertain the requirement for *de novo* protein synthesis in MADER expression, the following experiment was carried out. Mel JuSo cells were grown to confluence and treated for 2 hours with 10 ng/ml PMA in the presence or absence of cycloheximide (CHX). All treatments were performed in duplicate. Referring now to Figure 8, RNA from the treated cells was separated, blotted, and hybridized with probes detecting MADER, c-*fos* and GAPDH as described above. The basal levels of the three mRNAs are shown in lane 1.

As can be seen, preincubation with cycloheximide did not alter the basal level of MADER mRNA (lanes 3 and 4), but completely prevented its induction in response to PMA (lanes 6 and 7 as compared with lanes 9 and 10). In contrast, cycloheximide treatment resulted in an increase in c-*fos* mRNA (lanes 3 and 4) and in a superinduction following exposure to phorbol ester (lanes 6 and 7) as previously described (Treisman, R. (1985) *Cell* 42:889-902).

Accordingly, MADER gene expression is induced by mitogens and growth factors with mRNA

levels reaching a maximum of approximately 10 fold by 2 hours and returning to basal levels by 6 hours. This up-regulation is completely blocked by pretreatment with cycloheximide, indicating that it requires *de novo* protein synthesis. Genes that are expressed in the first hours following stimulation by growth factors or mitogens have been divided into two groups, the immediate early and the delayed early response genes (Pardee, A.B. (1989) *Science* 246:603-608; Nathans et al. (1988) *Cold Spring Harbor Symposia on Quantitative Biology* LIII:883-900). The immediate early response genes include transcription factors such as *c-fos* (Greenberg et al. (1984) *Nature* 311:433-438) and *c-myc* (Kelly et al. (1983) *Cell* 35:306) which are transcribed despite the presence of protein synthesis inhibitors. Transcription of the delayed early response genes is dependent on the products of the immediate early genes, and thus requires protein synthesis. The data presented here indicate that MADER is a delayed early response gene.

Example IV
Production of Anti-MADER Monoclonal Antibodies

Antigen Production:

MADER antigens were produced according to the new England Bio-Labs Protein Fusion and Purification System, using the pMAL-c2 expression vector. Particularly, a full length MADER cDNA clone of the Drop9 variant (Figure 1, SEQ ID NO.:___) was cloned into the pMAL expression vector and expressed in bacteria as a maltose-binding protein fusion product. The fusion protein was isolated from bacterial extracts using an amylose separation column. The subject MADER cDNA clone encodes a fusion protein referred to as the "Drop9" fusion protein.

Immunizations:

Female C57BL/6 x Balb/c F1 murine subjects received two subcutaneous challenges with 10 μg of the fusion protein in incomplete adjuvant 14 days apart. The subjects were then challenged with an intraperitoneal injection 14 days later using 2 μg of the fusion protein and 1 x 10^9 fixed *Bordetella pertussis* cells. 3 days later, the subjects were sacrificed and the spleens were removed.

Hybridoma Production:

B-cells obtained from the spleens were induced to fuse with P3x53Ag8.653 myeloma cells to form hybridomas. After selection in HAT medium, the resulting hybridomas were plated by limiting dilution and assayed for the production of antibodies capable of binding to the MADER antigen.

Screening:

ELISA plates were coated with the Drop9 fusion protein and with a control CD40 fusion protein in the same vector. Antibodies reacting only with the Drop9 fusion protein were cloned. The following monoclonal antibodies were obtained: 1C4; 1F12 (DSM Accession No. DSM ACC2251); 3B3; 4F2 (DSM Accession No.DSM ACC2250); 5B9; 5C3; 5H1 (DSM Accession No. DSM ACC2252); and 5H3.

Example V

Examination of MADER Expression in Human Cells Using Immunoperoxidase Staining

A panel of frozen tissue sections from normal human tissue and human malignant melanomas were examined to determine the level of expression of the MADER 55 kD protein. Cryocentrifuge preparations of cell lines or 5-μm frozen tissue sections were air

dried, fixed for 10 minutes in acetone and incubated for 1 hour at room temperature with the monoclonal antibodies produced in Example 4 in the form of tissue culture supernatants or as purified antibodies at 20 μg/ml. The slides were washed 3 times in phosphate buffered saline (PBS) and incubated for 1 hr with a peroxidase conjugated antiserum v. mouse immunoglobulin (Jackson ImmunoResearch Laboratories, West Grove PA). 3-amino-9-ethylcarbazole (0.25 mg/ml) and 0.003% H_2O_2 in 0.1 M acetate buffer pH 4.9 was used as substrate. After washing, the slides were either not counterstained or exposed briefly to hematoxylin. Monoclonal antibodies directed against the nevus/melanoma proteoglycan and against the leukocyte marker CD45 were used as positive controls and MOPC21 (IgG1, Sigma) as isotype control.

Examination of normal human tissue using immunoperoxidase staining of frozen sections revealed that most of the normal tissues examined did not express detectable levels of the MADER protein. Some staining was observed in sebaceous glands and sweat glands in occasional skin sections, but other structures and tissues including epidermis, mucosa of the gastrointestinal tract, vessels and smooth muscle, were each found to be negative.

Unlike the normal tissue sections, human malignant melanomas consistently showed strong nuclear staining. Specifically, two lymph node melanoma metastases, 538 and 408, exhibited strong nuclear staining with the anti-MADER monoclonal antibody 5H1. The anti-MADER monoclonal antibody 3B3 also exhibited strong nuclear staining in the melanoma cell line Mel Wei. As a control, the same regions were also stained with an antibody directed to the melanoma associated proteoglycan. The majority of tumor cells examined in both metastases were found to express easily detectable levels of MADER, while the surrounding

leukocytes were found to be negative. A similar pattern and level of expression of MADER was observed in nine different melanomas (primary as well as metastatic melanomas).

In contrast to the staining observed with the melanomas, epidermal melanocytes were not stained with the anti-MADER 5H1 antibody, and of six benign melanocytic tumors (nevi) examined, weak staining of isolated nests was seen in only one sample. Specifically, anti-MADER reactivity (as determined with the 5H1 monoclonal) of two different common acquired nevi (compound nevus 314 and dermal nevus 310) was found to be negligible. In both cases, anti-proteoglycan antibody was used on adjacent tissue sections to identify the nevus cells. No detectable anti-MADER reactivity was observed in five specimens of colorectal carcinomas indicating that high levels of MADER protein do not appear to be common to all types of malignant cells.

A panel of frozen tissue sections from human breast carcinoma tissue was examined as described above to determine the level of expression of the MADER 55 kD protein. In two out of ten samples examined, over-expression of MADER was observed, indicating that such over-expression is associated with breast carcinomas.

Furthermore, cytoplasmic, but not nuclear determinants of MADER were stained with the 1F12 anti-MADER antibody on paraffin sections treated as detailed below. In these cases, melanomas of early progression, but not metastases were stained, thus allowing further differentiation of tumor stages.

In the study, all incubations are carried out in a moist chamber at room temperature unless otherwise noted. Initially, tissue sections were treated with 0.25% trypsin (Sigma, St. Louis, Mo.) and 0.25% protease type 24 (Sigma) for 5 min.

The sections were then blocked with 0.2% BSA (Sigma A-3059) in PBS with sodium azide. The first incubation was carried out with monoclonal antibody as culture supernatant (1:2), for 1 hour. The sections were then carefully washed in PBS (the antibody was first drained off, and then slides were put into staining jars with PBS); incubation with PBS 4X (4 changes), for 5 min each.

Incubation for one hour with a secondary antibody (Jackson labs, 315-035-048, peroxidase-conjugated affinipure rabbit anti-mouse IgG + IgM) at a ratio of 1:200 in PBS, 0.2% BSA + azide. The wash step was repeated, and the substrate was incubated in substrate solution in staining jars, that were placed in a 37 °C water bath for between 7 to 15 min. The substrate (50 mg 3-amino-9-ethylcarbazole (Sigma) dissolved in 10 mL N,N-dimethylformamide (Sigma)) was then added to 0.1M acetate buffer ph 4.9, with 0.003% H_2O_2.

Accordingly, novel compositions and methods of detecting or treating malignant melanoma and other cancerous conditions have been described. Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

Deposits of Strains Useful in Practicing the Invention

A deposit of biologically pure cultures of the following strains was made with the German Collection of Microorganisms and Cell Cultures (DSMZ-Deutsche Sammlung Von Mikroorganismen und Zellkulturen GmbH), Mascheroder Weg 1b, D-38124 Braunschweig, Germany, under the provisions of the Budapest Treaty. The accession number indicated was assigned after successful viability testing, and the requisite fees were paid. Access to said cultures will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 122. All restriction on availability of said cultures to the public will be irrevocably removed upon the granting of a patent based upon the application. Moreover, the designated deposits will be maintained for a period of thirty (30) years from the date of deposit, or for five (5) years after the last request for the deposit; or for the enforceable life of the U.S. patent, whichever is longer. Should a culture become nonviable or be inadvertently destroyed, or, in the case of plasmid-containing strains, lose its plasmid, it will be replaced with a viable culture(s) of the same taxonomic description.

These deposits are provided merely as a convenience to those of skill in the art, and are not an admission that a deposit is required. The nucleic acid sequences of these plasmids, as well as the amino sequences of the polypeptides encoded thereby, are controlling in the event of any conflict with the description herein. A license may be required to

make, use, or sell the deposited materials, and no such license is hereby granted.

Strain	Deposit Date	DSM No.
1F12	19 December 1995	DSM ACC2251
4F2	19 December 1995	DSM ACC2250
5H1	19 December 1995	DSM ACC2252

Claims:

1. A monoclonal antibody capable of specifically binding to an approximately 55 kD MADER protein which is over-expressed in human malignant melanomas.

2. The monoclonal antibody of claim 1, wherein said antibody has an IgG1 isotype.

3. The monoclonal antibody of claim 1, wherein said antibody is secreted by a hybridoma selected from the group consisting of 1F12 (DSM Accession No. DSM ACC2251), 4F2 (DSM Accession No. DSM ACC2250), and 5H1 (DSM Accession No. DSM ACC2252).

4. The monoclonal antibody of claim 1, wherein said antibody is associated with a detectable label.

5. The monoclonal antibody of claim 4, wherein the detectable label is selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, dyes and metal ions.

6. A murine X murine hybridoma cell line that produces the monoclonal antibody of claim 1.

7. A method of detecting a cancerous condition, comprising:

(a) providing a tissue sample suspected of including cancerous cells;

(b) exposing said tissue sample to one or more monoclonal antibodies, wherein said antibodies are capable of specifically binding to an

approximately 55 kD MADER protein which is over-expressed in human malignant melanomas; and

(c) determining the presence or absence of bound antibodies on the tissue sample.

8. A method of detecting a cancerous condition, comprising:

(a) providing a tissue sample suspected of including cancerous cells;

(b) exposing the tissue sample to one or more monoclonal antibodies, wherein said monoclonal antibodies are capable of binding specifically to an approximately 55 kD MADER protein over-expressed in human malignant melanomas;

(c) exposing the tissue sample to a secondary antibody capable of binding said monoclonal antibodies; and

(d) determining the presence or absence of bound secondary antibodies on the tissue sample.

9. The method of claim 7 or 8, wherein the cancerous condition is a melanoma malignancy.

10. The method of claim 7 or 8, wherein the cancerous condition is a breast carcinoma.

11. The method of claim 7 or 8, wherein the tissue sample comprises a frozen tissue section.

12. The method of claim 7 or 8, wherein the tissue sample comprises a cell lysate.

13. The method of claim 7 or 8, wherein the tissue sample comprises a paraffin tissue section.

14. The method of claim 7 or 8, wherein the said one or more monoclonal antibodies are secreted by

a hybridoma selected from the group consisting of 1F12 (DSM Accession No. DSM ACC2251), 4F2 (DSM Accession No. DSM ACC2250), and 5H1 (DSM Accession No. DSM ACC2252).

15. The method of claim 14, wherein the said one or more monoclonal antibodies are associated with a detectable label.

16. The method of claim 15, wherein the detectable label is selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, dyes and metal ions.

17. The method of claim 8, wherein the secondary antibody is derived from anti-murine immunoglobulin antisera.

18. The method of claim 17, wherein the secondary antibody is associated with a detectable label.

19. The method of claim 18, wherein the detectable label is selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, dyes and metal ions.

20. A method of detecting a cancerous condition in a tissue sample containing cells, comprising visualizing the presence or absence of a translocation of MADER in said cells using fluorescence *in situ* hybridization (FISH).

21. A method of detecting a chromosomal rearrangement of MADER, comprising:

(a) providing immobilized chromosomal target DNA, wherein said target DNA has been obtained from a cell suspected of having undergone a MADER translocation event and said DNA has been rendered single-stranded;

(b) providing a single-stranded oligonucleotide probe having a nucleotide sequence complementary to a MADER nucleotide sequence present in the target DNA, wherein said probe comprises a moiety capable of direct or indirect visualization;

(c) incubating the target DNA and the oligonucleotide probe under hybridizing conditions to provide a target-probe hybrid; and

(d) visualizing to determine the site of the target-probe hybrid.

22. A method of detecting the presence of cancerous cells that over-express the MADER gene, comprising:

(a) obtaining a tissue sample suspected of including cancerous cells; and

(b) detecting the presence or absence of over-expression of the MADER gene.

23. The method of claim 22, wherein step (b) comprises: (1) preparing a cell lysate from the tissue sample, said lysate comprising ribonucleic acid (RNA) molecules; (2) extracting RNA from the lysate; (3) immobilizing the extracted RNA to a substrate; (4) providing a detectably labeled oligonucleotide probe, said probe comprising a nucleotide sequence having at least a 17 base pair region that is complementary to a region of an mRNA transcribed from the MADER gene; (5) incubating the immobilized RNA and the oligonucleotide probe under hybridizing conditions to provide a RNA-probe hybrid; (6) washing the substrate to remove any unbound probe; and (7) detecting the presence of any

labeled probe on the substrate to obtain a signal indicative of the presence or absence of over-expression of the MADER gene.

24. The method of claim 22, wherein step (b) entails *in situ* hybridization.

25. The method of claim 24, wherein step (b) comprises: (1) preparing a tissue section from the tissue sample, said section comprising ribonucleic acid (RNA) molecules; (2) immobilizing the tissue section to a substrate; (3) providing a detectably labeled oligonucleotide probe, said probe comprising a nucleotide sequence having at least a 17 base pair region that is complementary to a region of an mRNA transcribed from the MADER gene; (4) incubating the immobilized RNA and the oligonucleotide probe under hybridizing conditions to provide a RNA-probe hybrid; (5) washing the substrate to remove any unbound probe; and (6) detecting the presence of any labeled probe on the substrate to obtain a signal indicative of the presence or absence of over-expression of the MADER gene.

26. The method of any one of claims 23-25, wherein the oligonucleotide probe is labeled with a moiety selected from the group consisting of radioactive isotopes, fluorescers, chemiluminescers, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, dyes and metal ions.

27. A method of treating a cancerous condition in a subject, comprising administering a therapeutically effective amount of an antisense molecule capable of inhibiting the expression of the MADER gene.

28. A composition, comprising a MADER immunogen and a pharmaceutically acceptable vehicle.

29. The composition of claim 28, further comprising an adjuvant.

30. The composition of claim 28, wherein the MADER immunogen comprises a substantially full-length MADER polypeptide molecule.

31. The composition of claim 30, wherein the MADER polypeptide molecule has an amino acid sequence substantially homologous to the amino acid sequence depicted in Figure 1.

32. The composition of claim 30, wherein the MADER polypeptide molecule has an amino acid sequence substantially homologous to the amino acid sequence depicted in Figure 2.

33. The composition of claim 28, wherein the MADER immunogen comprises one or more peptide fragments of a MADER polypeptide molecule, said one or more fragments comprising a sequence of at least 6 amino acids.

34. The composition of claim 28, wherein the MADER immunogen comprises one or more deoxyribonucleic acid molecules comprising oligonucleotide sequences of at least about 18 to 30 base pairs that are substantially homologous to regions of the MADER gene.

35. The composition of claim 34, wherein the oligonucleotide sequences are substantially homologous to regions of the MADER nucleotide sequence depicted in Figure 1.

36. The composition of claim 34, wherein the oligonucleotide sequences are substantially homologous to regions of the MADER nucleotide sequence depicted in Figure 2.

37. A method of eliciting an immune response against a cell which over-expresses a MADER antigen, said method comprising administering a therapeutically effective amount of the composition of claim 49 to a subject suspected of having cells which over-express a MADER antigen.

38. A method of eliciting an immune response against a cell which over-expresses a MADER antigen, said method comprising administering a therapeutically effective amount of the composition of claim 50 to a subject suspected of having cells which over-express a MADER antigen.

FIG. 1A

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1     GlnArgAlaAsnLeuLeuSerTyrTyrGluThrPheIleGlnGlnGlyGlyAspAspVal
      CAGCGCGCCAACCTCCTTTCCTACTATGAGACCTTCATCCAGCAGGGAGGGGACGACGTG      60
21    GlnGlnLeuCysGluAlaGlyGluGluGluPheLeuGluIleMetAlaLeuValGlyMet
      CAGCAGCTGTGTGAGGCGGGTGAGGAGGAGTTTCTGGAGATCATGGCACTTGTGGGCATG     120
41    AlaThrLysProLeuHisValArgArgLeuGlnLysAlaLeuArgGluTrpAlaThrAsn
      GCCACCAAGCCCCTCCATGTCCGGCGCCTGCAGAAGGCACTGAGAGAGTGGGCCACCAAT     180
61    ProGlyLeuPheSerGlnProValProAlaValProValSerSerIleProLeuPheLys
      CCAGGGCTCTTCAGTCAACCAGTGCCTGCTGTTCCCGTCTCCAGCATCCCGCTCTTCAAG     240
81    IleSerGluThrAlaGlyThrArgArgGlySerMetSerAsnGlyHisGlySerProGly
      ATCTCTGAGACTGCGGGTACCCGGAAAGGGAGCATGAGCAATGGGCATGGCAGCCCAGGG     300
101   GluLysAlaGlySerAlaArgSerPheSerProLysSerProLeuGluLeuGlyGluLys
      GAAAAGGCAGGCAGTGCCCGCAGTTTTAGCCCCAAGAGCCCCCTTGAACTTGGAGAGAAG     360
121   LeuSerProLeuProGlyGlyProGlyAlaGlyAspProArgIleTrpProGlyArgSer
      CTATCACCACTGCCTGGGGGACCTGGGGCAGGGGACCCCCGGATCTGGCCAGGCCGGAGC     420
141   ThrProGluSerAspValGlyAlaGlyGlyGluGluGluAlaGlySerProProPheSer
      ACTCCAGAGTCGGACGTTGGGGCAGGAGGAGAAGAGGAGGCTGGCTCGCCCCCCTTCTCC     480
161   ProProAlaGlyGlyGlyValProGluGlyThrGlyAlaGlyGlyLeuAlaAlaGlyGly
      CCCCCTGCAGGGGGAGGAGTCCCTGAGGGGACTGGGGCTGGGGGGCTGGCAGCAGGTGGG     540
181   ThrGlyGlyGlyProAspArgLeuGluProGluMetValArgMetValValGluSerVal
      ACTGGGGGTGGTCCAGACCGACTGGAGCCAGAGATGGTACGCATGGTGGTGGAAAGTGTG     600
201   GluArgIlePheArgSerPheGlnGlyAspAlaGlyGluValThrSerLeuLeuLysLeu
      GAGAGGATCTTCCGGAGCTTCCAAGGGGATGCTGGGGAGGTCACATCCCTGCTAAAGCTG     660
221   AsnLysLysLeuAlaArgSerValGlyHisIlePheGluMetAspAspAsnAspSerGln
      AATAAGAAGCTGGCACGGAGCGTTGGGCACATCTTTGAGATGGATGATAATGACAGCCAG     720
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FIG. 1B

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                                        ..................................................
241     LysGluGluGluIleArgLysTyrSerIleIleTyrGlyArgPheAspSerLysArgArg
        AAGGAAGAGGAGATCCGCAAATACAGCATCATCTATGGCCGTTTCGACTCTAAGCGGCGG          780
761     GluGlyLysGlnLeuSerLeuHisGluLeuThrIleAsnGluAlaAlaAlaGlnPheCys
        GAGGGCAAGCAGCTCAGCCTGCACGAGCTCACCATCAACGAGGCTGCTGCCCAGTTCTGC          840
281     MetArgAspAsnThrLeuLeuLeuArgArgValGluLeuPheSerLeuSerArgGlnVal
        ATGAGGGACAACACACTCTTATTACGGAGAGTGGAGCTCTTCTCTTTGTCCCGCCAAGTA          900
301     AlaArgGluSerThrTyrLeuSerSerLeuLysGlySerArgLeuHisProGluGluLeu
        GCCCGAGAGAGCACCTACTTGTCCTCCTTGAAGGGCTCCAGGCTTCACCCTGAAGAACTG          960
321     GlyGlyProProLeuLysLysLeuLysGlnGluValGlyGluGlnSerHisProGluIle
        GGAGGCCCTCCACTGAAGAAGCTGAAACAAGAGGTTGGAGAACAGAGTCACCCTGAAATC         1020
341     GlnGlnProProProGlyProGluSerTyrValProProTyrArgProSerLeuGluGlu
        CAGCAGCCTCCCCCAGGCCCTGAGTCCTATGTACCCCCATACCGCCCCAGCCTGGAGGAG         1080
361     AspSerAlaSerLeuSerGlyGluSerLeuAspGlyHisLeuGlnAlaValGlySerCys
        GACAGCGCCAGCCTGTCTGGGGAGAGTCTGGATGGACATTTGCAGGCTGTGGGGTCATGT         1140
381     ProArgLeuThrProProProAlaAspLeuProLeuAlaLeuProAlaHisGlyLeuTrp
        CCAAGGCTGACGCCGCCCCCTGCTGACCTGCCTCTGGCATTGCCAGCCCATGGGCTATGG         1200
401     SerArgHisIleLeuGlnGlnThrLeuMetAspGluGlyLeuArgLeuAlaArgLeuVal
        AGCCGACACATCCTGCAGCAGACACTGATGGACGAGGGGCTGCGGCTCGCCCGCCTCGTC         1260
421     SerHisAspArgValGlyArgLeuSerProCysValProAlaLysProProLeuAlaGlu
        TCCCACGACCGCGTGGGCCGCCTCAGCCCCTGTGTGCCTGCGAAGCCACCTCTCGCAGAG         1320
441     PheGluGluGlyLeuLeuAspArgCysProAlaProGlyProHisProAlaLeuValGlu
        TTCGAGGAAGGGCTGCTGGACAGATGTCCTGCCCCAGGACCCCATCCCGCGCTGGTGGAG         1380
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FIG. 1C

461 GlyArgArgSerSerValLysValGluAlaGluAlaSerArgGln*

1440 GGTCGCAGGAGCAGCGTGAAAGTGGAGGCTGAGGCCAGCCGGCAGTGAGGGTTGGACTGG

1500 TGTCTTCAGACCCAGGACCTCAGACTTCTGGCTCACACAGACCCCCACGGCTCTCCATCCC

1560 CGGAATCTAGTCACAACCCTGGATCCTTCCTCTGCCCTTCTCCTGCCTCCCCACCTGCTC

1620 CATGGGCATAAGACTGTGGGGCTTCAAGCAATAACAAGCAGAGGCCTGGAGAGGAGGACAC

1680 AAGGAGGGTGCCGTGGTGCCCTCACCCCCTGCCCCAGAGCGAGGGGGCAAGGGACTCTGCCTCC

1740 AGGGCATCTGGGGTTTTCCCCTCCCTCACACAACACACTCCCCATTCTCTCTTTAGGTTTTGCA

1800 CCAGTGGTGTGAGCAGTTGGACTCAGTTTGGACAAGGGGGAGAAAGGGGGACTTCCCCTGG

1860 GAAGGTCCAGCTAAAAGTGGCAACATTTGCCCCCCAGAATTGGGGGCCTGGGAACACTGGA

1920 CCTGCTCCTTCTCCCCCTCCTCCCCGTTTTTGTGCTTCTGGTTTGTTTCTTTAATTAA

1980 TTTAACAAGTGCTGCAGTTTGCCCTCCCATTCTCCCCCAAGTCCTTTGCAATT

2040 TCTTCCCCTGCCCTACATAGGGGCGGTGGGTGTGGGATCCCCTTCACTGGCCCCCCTCGGGAG

2100 GCCTGGGTTGGACTCAGGGTCTCCTCCAGCTGGGGGGCTGGACCGCAGCACCTATCTGAGC

2160 AGTTAGAGCGTCTTTCTTTTCAGATTGTGTACAGTAGATTATTTATTTGTTATTTTGGA

ATAAAATTTATTTTATGGCTTAGGAAAAAAAA - 2192

FIG. 2A

```
1    GlnArgAlaAsnLeuLeuSerTyrTyrGluThrPheIleGlnGlnGlyGlyAspAspVal
     CAGCGCGCCAACCTCCTTTCCTACTATGAGACCTTCATCCAGCAGGGAGGGGACGACGTG                60
21   GlnGlnLeuCysGluAlaGlyGluGluGluPheLeuGluIleMetAlaLeuValGlyMet
     CAGCAGCTGTGTGAGGCGGGTGAGGAGGAGTTTCTGGAGATCATGGCACTTGTGGGCATG               120
41   AlaThrLysProLeuHisValArgArgLeuGlnLysAlaLeuArgGluTrpAlaThrAsn
     GCCACCAAGCCCCTCCATGTCCGGCGCCTGCAGAAGGCACTGAGAGAGTGGGCCACCAAT               180
61   ProGlyLeuPheSerGlnProValProAlaValProValSerSerIleProLeuPheLys
     CCAGGGCTCTTCAGTCAACCAGTGCCTGCTGTTCCCGTCTCCAGCATCCCGCTCTTCAAG               240
81   IleSerGluThrAlaGlyThrArgLysGlySerMetSerAsnGlyHisGlySerProGly
     ATCTCTGAGACTGCGGGTACCCGGAAAGGGAGCATGAGCAATGGGCATGGCAGCCCAGGG               300
101  GluLysAlaGlySerAlaArgSerPheSerProLysSerProLeuGluLeuGlyGluLys
     GAAAAGGCAGGCAGTGCCCGCAGTTTTAGCCCCAAGAGCCCCCTTGAACTTGGAGAGAAG               360
121  LeuSerProLeuProGlyGlyProGlyAlaGlyAspProArgIleTrpProGlyArgSer
     CTATCACCACTGCCTGGGGGACCTGGGGCAGGGGACCCCCGGATCTGGCCAGGCCGGAGC               420
141  ThrProGluSerAspValGlyAlaGlyGlyGluGluGluAlaGlySerProProPheSer
     ACTCCAGAGTCGGACGTTGGGGCAGGAGGAGAAGAGGAGGCTGGCTCGCCCCCCTTCTCC               480
161  ProProAlaGlyGlyGlyValProGluGlyThrGlyAlaGlyGlyLeuAlaAlaGlyGlu
     CCCCCTGCAGGGGGAGGAGTCCCTGAGGGGACTGGGGCTGGGGGGCTGGCAGCAGGTGGG               540
181  ThrGlyGlyGlyProAspArgLeuGluProGluMetValArgMetValValGluSerVal
     ACTGGGGGTGGTCCAGACCGACTGGAGCCAGAGATGGTACGCATGGTGGTGGAAAGTGTG               600
201  GluArgIlePheArgSerPheGlnGlyAspAlaGlyGluValThrSerLeuLeuArgLeu
     GAGAGGATCTTCCGGAGCTTCCAAGGGGATGCTGGGGAGGTCACATCCCTGCTAAAGCTG               660
221  AsnLysLysLeuAlaArgSerValGlyHisIlePheGluMetAspAsp X AspSerGlu
     AATAAGAAGCTGGCACGGAGCGTTGGGCACATCTTTGAGATGGATGATAATGACAGCCAG               720
```

FIG. 2B

```
                                        .....
241   LysGluGluGluIleArgLysTyrSerIleIleTyrGlyArgPheAspSerLysArgAsn
      AAGGAAGAGGAGATCCGCAAATACAGCATCATCTATGGCCGTTTCGACTCTAAGCGGCGG      780
761   GluGlyLysGlnLeuSerLeuHisGluLeuThrIleAsnGluAlaAlaAlaGlnPheCys
      GAGGGCAAGCAGCTCAGCCTGCACGAGCTCACCATCAACGAGGCTGCTGCCCAGTTCTGC      840
281   MetArgAspAsnThrLeuLeuLeuArgArgValGluLeuPheSerLeuSerArgGlnVal
      ATGAGGGACAACACACTCTTATTACGGAGAGTGGAGCTCTTCTCTTTGTCCCGCCAAGTA      900
301   AlaArgGluSerThrTyrLeuSerSerLeuLysGlySerArgLeuHisProGluGluLeu
      GCCCGAGAGAGCACCTACTTGTCCTCCTTGAAGGGCTCCAGGCTTCACCCTGAAGAACTG      960
321   GlyGlyProProLeuLysLysLeuLysGlnGluValGlyGluGlnSerHisProGluIle
      GGAGGCCCTCCACTGAAGAAGCTGAAACAAGAGGTTGGAGAACAGAGTCACCCTGAAATC     1020
341   GlnGlnProProProGlyProGluSerTyrValProProTyrArgProSerLeuGluGlu
      CAGCAGCCTCCCCCAGGCCCTGAGTCCTATGTACCCCCATACCGCCCCAGCCTGGAGGAG     1080
361   AspSerAlaSerLeuSerGlyGluSerLeuAspGlyHisLeuGln AlaValGlySerCys
      GACAGCGCCAGCCTGTCTGGGGAGAGTCTGGATGGACATTTGCAGGCTGTGGGGTCATGT     1140
381   ProArgLeuThrProProProAlaAspLeuProLeuAlaLeuProAlaHisGlyLeuTrp
      CCAAGGCTGACGCCGCCCCCTGCTGACCTGCCTCTGGCATTGCCAGCCCATGGGCTATGG     1200
401   SerArgHisIleLeuGlnGlnThrLeuMetAspGluGlyLeuArgLeuAlaArgLeuVal
      AGCCGACACATCCTGCAGCAGACACTGATGGACGAGGGGCTGCGGCTCGCCCGCCTCGTC     1260
421   SerHisAspArgValGlyArgLeuSerProCysValProAlaLysProProLeuAlaGlu
      TCCCACGACCGCGTGGGCCGCCTCAGCCCCTGTGTGCCTGCGAAGCCACCTCTCGCAGAG     1320
441   PheGluGluGlyLeuLeuAspArgCysProAlaProGlyProHisProAlaLeuValGlu
      TTCGAGGAAGGGCTGCTGGACAGATGTCCTGCCCCAGGACCCCATCCCGCGCTGGTGGAG     1380
```

FIG. 2C

```
61  GlyArgArgSerSerValLysValGluAlaGluAlaSerArgGln*                    1440
GGTCGCAGGAGCAGCGTGAAAGTGGAGGCTGAGGCCAGCCGGCAGTGAGGGTTGGACTGG      1500
TGTCTTCAGACCCAGGACCTCAGACTTCTGGCTCACACAGACCCCCACGCTCTCCATCCC      1560
CGGAATCTAGTCACACAACCCTGGATCCTTCCTCTGCCCTTCTCCTGCCTCCCCACCTGCTC    1620
CATGGGCATAAGACTGTGGGGCTTCAAGCAATAACAAGCAGAGGCCTGGAGAGAGGACAC     1680
AAGGAGGGTGCGTGGTGCCCTCACCCCTGCCCAGAGCGAGGGGGGCAAGGACTCTGCCTCC    1740
AGGGCATCTGGGGTTTTCCCCTCCTCACACAACACACTCCCATTCTCTCTTTAGGTTTGCA     1800
CCAGTGGTGTGAGCAGTTGGACTCAGTTTGGACAAGGGGGAGAAGGGGGACTTCCCTGG      1860
GAAGGTCCAGCTAAAAGTGGCAACATTTGCCCCCAGAATTGGGGGCCTGGGAACACTGGA     1920
CCTGCTCCTTCTCCCCTCTCCTTCCCCGTTTTTTGTGCTTCTGGTTTGTTTCTTTAATTAA    1980
TTTAACAAGTGCTGCAGTTTGCCCTCCCATTCCCCCATCTATCCCCCAAGTCCTTTGCAATT    2040
TCTTCCCTGCCCTACATAGGGGCGGTGGGTGTGGGATCCCTTCACTGGCCCCCTCGGGAG     2100
GCCTGGGTTGGACTCAGGGTCTCCTCCAGCTGGGGGCTGGACCGCAGCCACCTATCTGAGC    2160
AGTTAGAGCGTCTTTCTTTTCAGATTGTGTACAGTAGATTATTTATTTGTTATTTTTGGA
ATAAAATTTATTTATGGCTTAGGAAAAAAAA - 2192
```

FIG. 3A

```
   1 GACAGAGGCG CGGAGGCTCG GAGAGAGAAG ACGTGGAGGG AGGGACAGAG CCTGGACAGC
  61 GGTGGACACG GCATCGTGCG CGGGGAAGAG GGCAGCACGC AGCAGGCGCC GAGCGGCGGG
 121 CACCGGGAAG GGCAGCCCGG GTGATCTCCG GCCGTCCATG CACAGAGCGC CTTCCCCCAC
 181 AGCCGAGCAG CCGCCGGGCG GAGGGGACAG CGCCCGCCGG ACCCTGCAGC CCAGACTCAA
 241 GCCCAGTGCC CGAGCCATGG CACTGCCTCG CACGCTGGGG GAGCTGCAGC TGTACCGGGT
 301 CCTGCAGCGC GCCAACCTCC TTTCCTACTA TGAGACCTTC ATCCAGCAGG GAGGGGACGA
 361 CGTGCAGCAG CTGTGTGAGG CGGGTGAGGA GGAGTTTCTG GAGATCATGG CACTTGTGGG
 421 CATGGCCACC AAGCCCCTCC ATGTCCGGCG CCTGCAGAAG GCACTGAGAG AGTGGGCCAC
 481 CAATCCAGGG CTCTTCAGTC AACCAGTGCC TGCTGTTCCC GTCTCCAGCA TCCCGCTCTT
 541 CAAGATCTCT GAGACTGCGG GTACCCGGAA AGGGAGCATG AGCAATGGGC ATGGCAGCCC
 601 AGGGGAAAAG GCAGGCAGTG CCCGCAGTTT TAGCCCCAAG AGCCCCCTTG AACTTGGAGA
 661 GAAGCTATCA CCACTGCCTG GGGGACCTGG GGCAGGGGAC CCCCGGATCT GGCCAGGCCG
 721 GAGCACTCCA GAGTCGGACG TTGGGGCAGG AGGAGAAGAG GAGGCTGGCT CGCCCCCCTT
 781 CTCCCCCCCT GCAGGGGAG GAGTCCCTGA GGGGACTGGG GCTGGGGGGC TGGCAGCAGG
 841 TGGGACTGGG GGTGGTCCAG ACCGACTGGA GCCAGAGATG GTACGCATGG TGGTGGAAAG
 901 TGTGGAGAGG ATCTTCCGGA GCTTCCCAAG GGGGGATGCT GGGGAGGTCA CATCCCTGCT
 961 AAAGCTGAAT AAGAAGCTGG CACGGAGCGT TGGGCACATC TTTGAGATGG ATGATAATGA
1021 CAGCCAGAAG GAAGAGGAGA TCCGCAAATA CAGCATCATC TATGGCCGTT TCGACTCTAA
1081 GCGGCGGGAG GGCAAGCAGC TCAGCCTGCA CGAGCTCACC ATCAACGAGG CTGCTGCCCA
1141 GTTCTGCATG AGGGACAACA CGCTCTTATT ACGGAGAGTG GAGCTCTTCT CTTTGTCCCG
1201 CCAAGTAGCC CGAGAGAGCA CCTACTTGTC CTCCTTGAAG GGCTCCAGGC TTCACCCTGA
1261 AGAACTGGGA GGCCCTCCAC TGAAGAAGCT GAAACAAGAG GTTGGAGAAC AGAGTCACCC
1321 TGAAATCCAG CAGCCTCCCC CAGGCCCTGA GTCCTATGTA CCCCCATACC GCCCCAGCCT
1381 GGAGGAGGAC AGCGCCAGCC TGTCTGGGGA GAGTCTGGAT GGACATTTGC AGGCTGTGGG
1441 GTCATGTCCA AGGCTGACGC CGCCCCCTGC TGACCTGCCT CTGGCATTGC CAGCCCATGG
1501 GCTATGGAGC CGACACATCC TGCAGCAGAC ACTGATGGAC GAGGGGCTGC GGCTCGCCCG
1561 CCTCGTCTCC CACGACCGCG TGGGCCGCCT CAGCCCCTGT GTGCCTGCGA AGCCACCTCT
1621 CGCAGAGTTC GAGGAAGGGC TGCTGGACAG ATGTCCTGCC CCAGGACCCC ATCCCGCGCT
1681 GGTGGAGGGT CGCAGGAGCA GCGTGAAAGT GGAGGCTGAG GCCAGCCGGC AGTGA
```

FIG. 3B

```
1    MHRAPSPTAEQPPGGGDSARRTLQPRLKPSARAMALPRTLGELQLYRVLQ
51   RANLLSYYETFIQQQGGDDVQQLCEAGEEEFLEIMALVGMATKPLHVRRLQ
101  KALREWATNPGLFSQPVPAVPVSSIPLFKISETAGTRKGSMSNGHGSPGE
151  KAGSARSFSPKSPLELGEKLSPLPGGPGAGDPRIWPGRSTPESDVGAGGE
201  EEAGSPPFSPPAGGGVPEGTGAGGLAAGGTGGGPDRLEPEMVRMVVESVE
251  RIFRSFPRGDAGEVTSLLKLNKKLARSVGHIFEMDDNDSQKEEEIRKYSI
301  IYGRFDSKRREGKQLSLHELTINEAAAQFCMRDNTLLLRRVELFSLSRQV
351  ARESTYLSSLKGSRLHPEELGGPPLKKLKQEVGEQSHPEIQQPPPGPESY
401  VPPYRPSLEEDSASLSGESLDGHLQAVGSCPRLTPPPADLPLALPAHGLW
451  SRHILQQTLMDEGLRLARLVSHDRVGRLSPCVPAKPPLAEFEEGLLDRCP
501  APGPHPALVEGRRSSVKVEAEASRQ

1    MHRAPSPTAEQPPGGGDSARRTLQPRLKPSARAMALPRTLGELQLYRVLQ
51   RANLLSYYETFIQQQGGDDVQQLCEAGEEEFLEIMALVGMATKPLHVRRLQ
101  KALREWATNPGLFSQPVPAVPVSSIPLFKISETAGTRKGSMSNGHGSPGE
151  KAGSARSFSPKSPLELGEKLSPLPGGPGAGDPRIWPGRSTPESDVGAGGE
201  EEAGSPPFSPPAGGGVPEGTGAGGLAAGGTGGGPDRLEPEMVRMVVESVE
251  RIFRSFPRGDAGEVTSLLKLNKKLARSVGHIFEMDDNDSQKEEEIRKYSI
301  IYGRFDSKRREGKQLSLHELTINEAAAQF
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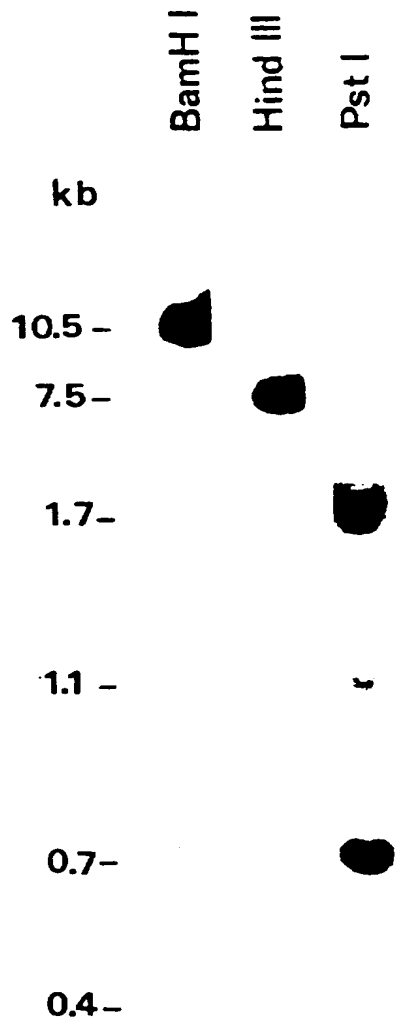

FIG. 4

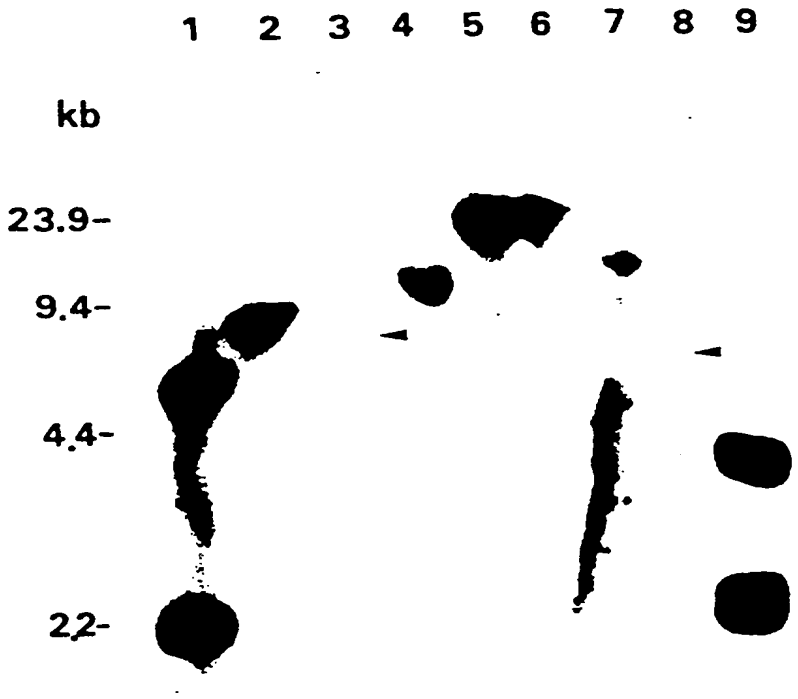

FIG. 5

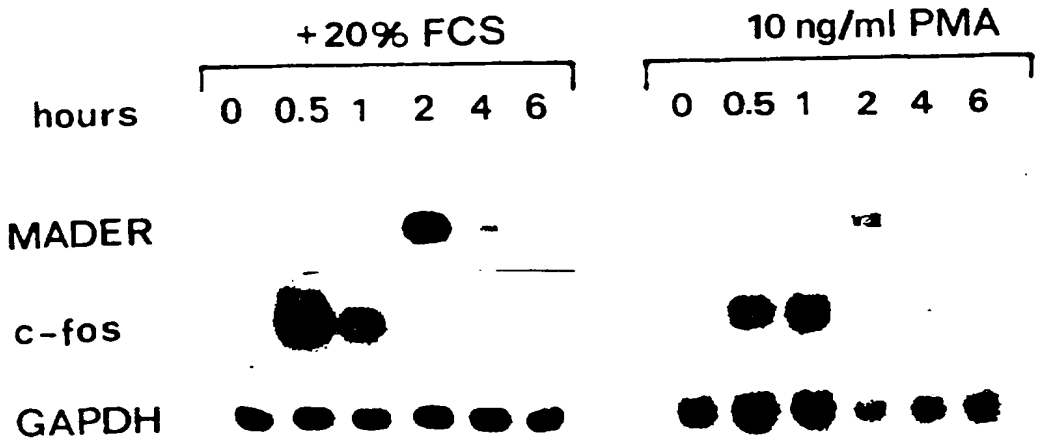

FIG. 6

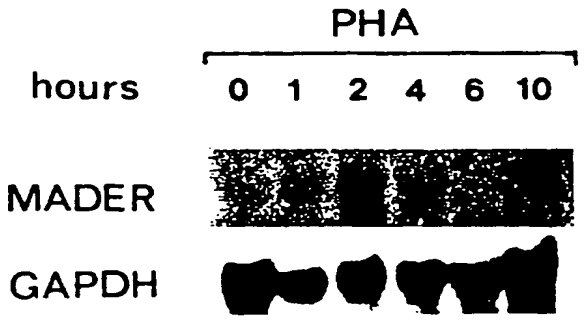
PHA
hours
0 1 2 4 6 10
MADER
GAPDH

FIG. 7

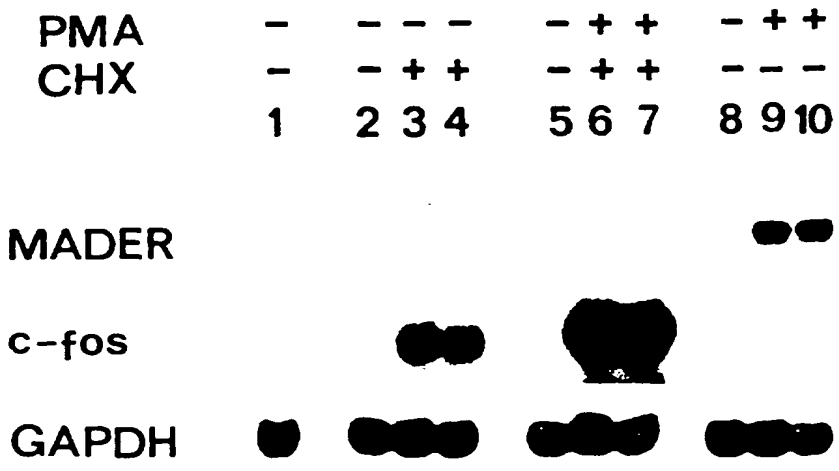
PMA
CHX
- - - - - + + - + +
- - + + - + + - - -
1 2 3 4 5 6 7 8 9 10
MADER
c-fos
GAPDH

FIG. 8

INTERNATIONAL SEARCH REPORT

Internatic Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/32 G01N33/574 C07K14/82 C12Q1/68 G01N33/577

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B. FIELDS SEARCHED

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,X	DATABASE CANCERLIT AN-95604942, 1994 KIRSCH, K. ET AL.: "MADER, a novel melanoma-associated delayed early response gene, constitutively expressed in tumor cells." XP002034502 see abstract	1-38
X	& PROC. ANNU. MEET. ASSOC. CANCER RES., vol. 35, 1994, page a3257 KIRSCH, K., RIETHMULLER G. AND JOHNSON, J.P.: see the whole document --- -/--	1-38

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8 July 1997	16.07.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hoekstra, S

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 97/01586

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL DATABASE s31927, 6 January 1995 HILLIER L. ET A.: XP002034503 See database entry & unpublished	1-38
P,X	ONCOGENE, vol. 12, 1996, pages 963-972, XP002034498 KIRSCH, K. ET AL.: "Mader, a novel nuclear protein overexpressed in human melanomas" see the whole document & DATABASE EMBL DATABASE X70991, 24 February 1993 KIRSCH K. ET AL: See Database entry	1-38
P,X	MOLECULAR AND CELLULAR BIOLOGY, vol. 16, no. 7, July 1996, pages 3545-3553, XP002034499 SVAREN, J. ET AL.: "NAB2, a corepressor of NGFI-A (Egr-1) and Krox20, is induced by proliferative and differentiative stimuli" cited in the application see the whole document	1-38
A	PROC. NATL. ACAD. SCI. USA, vol. 92, July 1995, pages 6873-6877, XP002034500 RUSSO M.W. ET AL.: "Identification of NAB1, a repressor of NGFI-A and Krox20 mediated transcription." cited in the application see the whole document	1-38
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A	PROC. NATL. ACAD. SCI USA, vol. 86, December 1989, pages 9891-9895, XP002034501 LEHMANN, J.M. ET AL.: "MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily" cited in the application see the whole document ---	1-38
A	MOLECULAR AND CELLULAR BIOLOGY, vol. 12, no. 9, 1 September 1992, pages 3919-3929, XP000569563 LANAHAN A ET AL: "GROWTH FACTOR-INDUCED DELAYED EARLY RESPONSE GENES" -----	1-38

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(54) Title: COMPOSITIONS AND METHODS USEFUL IN THE DETECTION AND/OR TREATMENT OF CANCEROUS CONDITIONS

(57) Abstract

Diagnostic and therapeutic compositions and methods which target a melanoma associated delayed early response (MADER) gene and its expression products are described. Specifically, the invention relates to the production, characterization and use of monoclonal antibodies capable of specifically binding to an approximately 55 kD MADER protein which is overexpressed in human malignant melanomas and other human cancerous tissue. Such antibodies are able to detect overexpressed MADER in cultured cells and frozen or paraffin-embedded sections of human biopsy material. The MADER protein, fragments or analogs thereof, or its gene in a vector suitable for a DNA vaccine, are employed as anti-cancer immunogens in the immunotherapeutic treatment of malignant melanoma and other cancerous conditions. Similarly, MADER polynucleotides are used herein in various cytological methods for detecting cells which overexpress MADER. MADER mRNAs are used as targets in antisense and ribozyme therapies directed at inhibiting the expression of MADER in a treated subject.

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8 July 1997

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16. 07. 97

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL DATABASE s31927, 6 January 1995 HILLIER L. ET A.: XP002034503 See database entry & unpublished	1-38

P,X	ONCOGENE, vol. 12, 1996, pages 963-972, XP002034498 KIRSCH, K. ET AL.: "Mader, a novel nuclear protein overexpressed in human melanomas" see the whole document & DATABASE EMBL DATABASE X70991, 24 February 1993 KIRSCH K. ET AL: See Database entry	1-38

P,X	MOLECULAR AND CELLULAR BIOLOGY, vol. 16, no. 7, July 1996, pages 3545-3553, XP002034499 SVAREN, J. ET AL.: "NAB2, a corepressor of NGFI-A (Egr-1) and Krox20, is induced by proliferative and differentiative stimuli" cited in the application see the whole document	1-38

A	PROC. NATL. ACAD. SCI. USA, vol. 92, July 1995, pages 6873-6877, XP002034500 RUSSO M.W. ET AL.: "Identification of NAB1, a repressor of NGFI-A and Krox20 mediated transcription." cited in the application see the whole document	1-38

A	DATABASE EMBL DATABASE R20420, 23 April 1995 HILLIER L. ET AL.: "The WasU-Merck EST Project" XP002034504 See Database entry & unpublished	1-38

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INTERNATIONAL SEARCH REPORT

Internatic Application No
PCT/US 97/01586

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE EMBL DATABASE H14844, 3 July 1995 HILLIER L. ET AL.: "The WashU-Merck EST Project" XP002034505 See Database entry & unpublished	1-38
A	PROC. NATL. ACAD. SCI USA, vol. 86, December 1989, pages 9891-9895, XP002034501 LEHMANN, J.M. ET AL.: "MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily" cited in the application see the whole document	1-38
A	MOLECULAR AND CELLULAR BIOLOGY, vol. 12, no. 9, 1 September 1992, pages 3919-3929, XP000569563 LANAHAN A ET AL: "GROWTH FACTOR-INDUCED DELAYED EARLY RESPONSE GENES"	1-38

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REVISED VERSION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01586

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 530/387.7, 388.1; 435/7.1, 330

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/387.7, 388.1; 435/7.1, 330

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, EMBASE, CAPLUS
search terms: melanoma, antibod?, mader, diagnosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	LEHMANN et al. MUC18, a marker of tumor progression in human melanoma, shows sequence similarity to the neural cell adhesion molecules of the immunoglobulin superfamily. Proc. Natl. Acad. Sci. USA. December 1989, Vol. 86, pages 9891-9895, see entire document.	1, 4, 5, 7, 9, 11, _____ 2, 3, 6, 12-16
Y	KIRSCH et al. MADER, a novel melanoma associated delayed early response gene, constitutively expressed in tumor cells. Proc. Am. Assoc. Cancer Res. Annual Mtg. 1994, Vol. 35, Abstract No. 3257, page 547, see entire abstract.	1-7, 9 and 11-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 AUGUST 1997	10 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SUSAN UNGAR Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01586

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KIRSCH et al. Mader: a novel nuclear protein over expressed in human melanomas. Oncogene. 1996, Vol 12, pages 963-971, see entire document.	1-7, 9 and 11-16
Y	RUITER et al. Immunohistochemistry in the Evaluation of Melanocytic Tumors. Seminars in Diagnostic Pathology. February 1993, Vol 10, No 1, pages 76-91, see especially pages 76-78, 82, and 84-86.	1-7, 9 and 11-16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01586

Box I Observations where certain claims were f und unsearchable (Continuation f item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-7 and 9-16 as they are drawn to the invention of claim 7.

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/01586

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6): C12N 5/00; G01N 33/53
BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s) 1-7 and 9-16, drawn to a monoclonal antibody capable of specifically binding to an approximately 55 kD MADER protein and a method of detecting a cancerous condition with one or more antibodies that specifically bind to MADER protein.
Group II, claim(s) 8, 17-19 and 9-16, drawn to a method of detecting a cancerous condition with one or more antibodies that specifically bind to the MADER protein and a secondary antibody capable of binding said monoclonal antibodies.
Group III, claim(s) 20, drawn to a method of detecting a cancerous condition using an in situ hybridization assay for MADER.
Group IV, claim(s) 21, drawn to a method of detecting chromosomal rearrangement of MADER.
Group V, claim(s) 22-26, drawn to a method of detecting the presence of cancerous cells that over-express the MADER gene.
Group VI, claim(s) 27, drawn to a method of treating a cancerous condition by administering an antisense molecule capable of inhibiting the expression of the MADER gene.
Group VII, claim(s) 28-36, drawn to a composition comprising MADER immunogen and a pharmaceutically acceptable vehicle.
Group VIII, claim(s) 37-38, drawn to a method of eliciting an immune response against a cell which over-expresses a MADER antigen.
The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
(a) The claimed inventions of Group I and II are drawn to different methods of use of the antibodies recited in claims of Group I. Lack of unity of invention may be held when claims are drawn to more than one use of a claimed product (antibodies) or compositions comprising the antibodies. See 37 CFR 1.475 (d).
(b) Lack of unity of the inventions of Groups III-VIII may be held because these groups do not contain the special technical feature of monoclonal antibodies disclosed in Group I.